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# An Investigation of the Role Played by Catecholamines in the Metabolic Acclimation of Sheep to Cold

by

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## Abstract

Studies were carried out to determine the effect of chronic moderate and intermittent cold exposure on plasma catecholamine levels in sheep, to determine in warm- and cold-acclimated sheep the calorigenic response to adrenaline (A) and and noradrenaline (NA) intrajugular infusions at doses ranging from 0.15 to 0.90 ug/Kg/min and to assess the tissue turnover rate of NA in warm- and cold-acclimated sheep. The plasma NA and A levels were consistently elevated 2 to 3 fold,respectively, in sheep exposed to chronic moderate (8-9°C) cold for 44 days. For a 50 day period, between the hours of 2200 and 800, the intermittent cold exposed sheep were subjected to low ambient temperatures (-2-4°C). Plasma NA concentration, measured in the intermittent cold exposed sheep during the warm (19-24°C) daytime period, was at least 3 times that of control sheep which were held at 22-26°C. Intermittent cold exposure did not result in a significant elevation in thermoneutral plasma A concentration.

During A infusions of 2.5 h duration the heat production of sheep acclimated to 19-24°C for 3 to 8 weeks increased 35% and the heat production response to A of sheep acclimated to 8-13°C (53% increase in heat production) was significantly ($P<0.01$) greater than that of the warm-acclimated sheep. Noradrenaline infusion caused a similar maximum 30% increase in heat production in the warm- and cold-acclimated sheep. Shivering was rarely observed

during catecholamine infusions. The thermoneutral heat production of cold-acclimated sheep was greater than that of warm-acclimated sheep by 2% per °C difference in acclimation temperatures.

The heat production response of sheep to cold may involve some catecholamine stimulated heat production since both A and NA were found to be calorigenic in warm-acclimated sheep and plasma concentration of catecholamines was found to be elevated throughout prolonged periods of cold exposure. Adrenaline calorigenesis in sheep may take on an even more important thermogenic role following cold acclimation when the sensitivity of the animal to this hormone increases.

As determined by measuring the disappearance of injected $^{3}$H-noradrenaline from tissue stores, the turnover rate of NA in the leg skin of cold-acclimated sheep was three times that found in warm-acclimated sheep but cold acclimation did not significantly affect gut tissue NA turnover rate.

The studies show that plasma catecholamine concentrations remain elevated during a period of long term cold exposure and it is possible that the demonstrated calorigenic effect of catecholamines in sheep could contribute to the increased thermoneutral heat production and cold induced heat production of cold-acclimated sheep.

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# I. Introduction

The metabolic acclimation of adult sheep and cattle to low environmental temperatures is characterized by an elevation in thermoneutral heat production (Webster *et al*.1969b; Young 1975), an enhanced ability to raise heat production during very severe cold exposure (Webster *et al*.1969b) and possibly a replacement of shivering with nonshivering thermogenesis (Slee 1972; Young 1975). There is also evidence (Slee 1972; Webster *et al*.1969b) that the nature of acclimation is dependent on the type of cold exposure. Factors which mediate the metabolic acclimation of ruminants to cold are unknown but a role played by both the thyroid and sympatheticoadrenal medullary systems has been suggested (Young 1975). Although elevated plasma noradrenaline and adrenaline levels are found in acutely cold exposed sheep (Thompson *et al*.1978) and plasma noradrenaline level has been shown to be elevated in sheared sheep exposed for four weeks to moderate cold (Christopherson *et al*.1978), a role played by the catecholamines in mediating the metabolic acclimation of sheep to cold has been disputed because the results of Webster *et al*.(1969a) indicate that catecholamines are not calorigenic in sheep.

Another response of sheep to chronic cold exposure is an increase in reticulorumen motility (Westra and Christopherson 1976) which apparently contributes to reduced reticulorumen retention time and reduced feed digestibility (Kennedy *et al*.1977). The effect of chronic cold exposure on

reticulorumen retention time and feed digestibility could only be partially attributed to a change in thyroid function and a contribution by catecholamines was implicated.

The aim of the present study was to assess, by measuring plasma catecholamine levels and noradrenaline turnover rate, the response of the sympathetic nervous system of sheep to long term cold exposure and to re-investigate the possibility that catecholamines are calorigenic in adult sheep. In Study 1 plasma catecholamine levels were compared in sheep subjected to control, moderate cold or intermittent cold temperature treatments. Cortisol metabolism and thermoneutral heat production were also measured during the period of cold exposure such that the nature of acclimation could be compared between treatment groups. In Study 2 the calorigenic effects of adrenaline and noradrenaline were examined in sheep acclimated for three to eight weeks to either warm or moderately cold temperatures. In Study 3 a radioisotope technique previously used with small mammals for the assessment of noradrenaline turnover rate was applied to sheep acclimated for eight weeks to warm or moderately cold temperatures.

In addition, because cold acclimation of sheep results in digestive as well as metabolic alterations, when possible, digestive responses to cold and catecholamines were studied. The rumen motility response to intravenous infusions of adrenaline and noradrenaline was studied in Study 2 and gut tissue catecholamine levels and

noradrenaline turnover rate as well as gastrointestinal organ weights of warm-and cold-acclimated sheep were compared in Study 3.

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## II. Study 1

## Endocrine and Metabolic Changes in Sheep Associated with Acclimation to Constant or Intermittent Cold Exposure

### A. Abstract

The plasma noradrenaline (NA), adrenaline (A) and dopamine (DA) levels and cortisol metabolism were studied in three groups of three sheared year-old wethers exposed for 50 d to either 22-26°C, 8-9°C or to 19-24°C from 900 h to 2100 h and -2-4°C from 2200 to 800 h. Plasma samples for catecholamine and cortisol determination were taken once per week, three h following the daily 800 h and 1700 h feeding periods. Midway and near the end of the 50 d exposure period cortisol entry rate and metabolic clearance rate (cMCR) were estimated using a $^{3}$H-cortisol continuous infusion technique and respired gas analysis was used to estimate the heat production (HP) of all sheep when fasted 51 h and exposed to thermoneutral temperatures.

An increase in thermoneutral HP following 27-30 d of cold exposure indicated that sheep of both cold treatment groups had developed some form of cold-acclimation. Exposure of sheep to 8-9°C resulted in a three- and two-fold increase in plasma NA and A levels, respectively, and the elevated levels were maintained for the duration of the trial. Nightly cold exposure of the intermittent cold exposed sheep initially resulted in greatly elevated daytime plasma NA levels but these levels tended to decrease over time.

Intermittent cold exposure did not significantly affect plasma A level and neither of the two cold treatments significantly affected plasma DA level. Chronic exposure to 8-9°C but not intermittent cold exposure caused an increase in cMCR and plasma cortisol level. Cortisol entry rate did not differ significantly between treatment groups.

## B. Introduction

The acclimatization of sheep to winter conditions and the laboratory acclimation of sheep to constant low temperature both result in elevated thermoneutral heat production (Webster *et al*.1969b). Low temperature acclimation results in an enhanced ability of sheep to respond metabolically to a thermally threatening environment (-30°C for 3 h) while winter acclimatization seems to be characterized by a supressed metabolic response to acute cold (Webster *et al*.1969b). It is possible that the intermittancy of cold stress in a winter environment is an effector to which sheep habituate as do sheep exposed to repeated short cold shocks (Slee 1972).

The endocrinology of cold-acclimation in sheep was first studied by Webster *et al*.(1969a). Urinary excretion of catecholamines was found to be inversely related to the temperature of acclimation. Plasma concentrations of adrenaline (A) and noradrenaline (NA) have since been found to be elevated following a four week exposure of shorn sheep to 2-5 °C (Christopherson *et al*.1978). The plasma catecholamine levels of sheep exposed to low temperature for a period longer than four weeks or exposed to intermittent cold and warm temperatures have not been studied. Increases in cortisol metabolic clearance rate, entry rate and plasma level have been observed upon acute cold exposure of sheep (Panaretto and Vickery 1970, 1972) but these endocrine parameters have not been studied in sheep under conditions

of prolonged constant or intermittent cold exposure treatments. The present study was designed to determine the effects of long term constant or intermittent cold exposure on plasma catecholamine and cortisol levels as well as cortisol metabolic clearance and entry rate in sheep. In addition, thermoneutral HP, heart rate (HR) and rectal temperature (RT) were monitored as indicators of the type of acclimation developed under the different long term temperature regimes.

## C. Material and Methods

Year old wethers averaging 39 kg were shorn once weekly to a fleece depth of 4-6 mm and were housed in individual metabolism crates in either a holding room or controlled environmental chambers. Prior to cold exposure all animals were held for 28 days at 22-29°C such that pre-cold parameters could be assessed. For the 50 day exposure period three control (C) sheep were held at 22-26°C, three chronic cold exposed (CC) sheep were held at 8-9°C and three intermittent cold exposed (IC) sheep were held in an environment where ambient temperature ranged from -2 to 4°C from 2200 h to 800 h and from 19 to 24°C from 900 h to 2100 h. The two cold regimes were designed to provide similar degree hours per day below the lower critical temperature of shorn sheep and temperatures of 19 - 24°C were within the thermoneutral zone of shorn sheep. Room lighting was continuous and the sheep were fed daily at 800 h and 1700 h. To ensure that all sheep would maintain body weight throughout the experiment, sheep in a thermoneutral environment received 550 g of alfalfa pellets and sheep in the colder environments received 650 g at each feeding. Fresh water and cobalt-iodized salt were offered *ad libitum*.

Blood samples were taken via indwelling jugular cannulae at 1100 h and 2000 h on days 14 and 15 of the pre-exposure period and on days 1, 2, 9, 16, 23, 37 and 44 of the differential temperature exposure periods. To minimize emotional disturbance of the sheep at the time of

blood sampling the cannulae were inserted at least 1 day prior to sampling. At least 1 h prior to sampling the cannulae were extended through portholes so that samples could be taken without laboratory personnel entering the animal rooms. The 10 ml blood samples were collected in 10 ml disposable syringes containing 200 ul glutathione/EGTA preservative solution as described by Peuler and Johnson (1977). A small volume of blood was set aside for hematocrit determination and the remainder was transferred to glass centrifuge tubes pre-chilled in an ice bath. Following immediate centrifugation of the blood at 16,000 X G and 4°C for 5 min the plasma was transferred to small plastic vials and was quickly frozen on dry ice and stored at -40°C.

Plasma concentrations of NA, A and dopamine (DA) were measured by the radioenzymatic assay technique of Peuler and Johnson (1977) with some minor modifications. Plasma samples of 100 ul volume were analyzed and the acetic acid/ethanol extracts were applied to Whatman multichannel silica gel plates (18 channels, 250 um thickness). The silica gel comprising the methoxytyramine zone was treated as recommended by the Upjohn Co. (Kalamazoo, Michigan) in the Cat-A-Kit procedure manual. A Mark III Liquid Scintillation Spectrometer (Searle Analytic Inc., Des Plaines, Ill.) was used to measure radioactivity.

During days 2-5 and 35-38 of the 50 day exposure period cortisol metabolic clearance rate (cMCR) and entry rate were estimated using the $^{3}$H-cortisol continuous infusion

technique of Marple and Cassens (1973). At least 3 days prior to a cortisol metabolism trial each sheep was implanted with an infusion catheter 25 cm into the right jugular vein and a sampling catheter 15 cm into the left jugular vein. At 1000 h on the day of the trial the catheters which were 3 m in length were extended to a sampling station outside the animal room. Commencing at 1200 h, 1,2-$^{3}$H-cortisol (Amersham, specific activity = 41 Ci/mM) in sterile saline containing 2% ethanol was infused (1.0 ml/min) at a dose rate of 0.5 $u$Ci/min for 4 h. A lambda pump (Model 1300, Harvard Apparatus Co., Mass.) was used to pump the infusate from a reservoir held in an ice-water bath. Five blood samples each of 7 ml volume, taken into heparinized syringes during the last 40 min of infusion, were centrifuged and the plasma was stored at -20°C until equilibrium concentration of labelled and unlabelled cortisol could be determined. Plasma concentration of $^{3}$H-cortisol was determined by extracting 400 $u$l plasma with 4 ml ethanol. To estimate recovery 3000 d.p.m. of 4-$^{14}$C-cortisol (Amersham, specific activity = 53 mCi/mM) was added to the plasma prior to extraction. The ethanol extract was dried under nitrogen and the sample was reconstituted with 200 $u$l ethanol:benzene (1:10) containing 2 ng/ml carrier cortisol. The sample was then applied to a 250 $u$m thick flourescent silica gel plate and the plate was developed for 20 min in a benzene:acetone (50:50) solvent system. ACS scintillation fluid (Amersham) was used for dual

label counting in the Mark III Spectrometer. Radioimmunoassay of plasma cortisol was performed by the use of a Micromedic D-0302 Cortisol Autopak (Micromedic Systems, Horsham, Pa.) in conjunction with a gamma counter (Biogamma; Beckman Instruments Inc., Fullerton, Calif.) for the determination of $^{125}I$. The cMCR (l/min) was calculated as the ratio of infusion rate of $^{3}H$-cortisol ($\mu$Ci/min) to mean equilibrium plasma concentration of $^{3}H$-cortisol ($\mu$Ci/l). Cortisol entry rate was the product of cMCR and plasma cortisol concentration ($\mu$g/l).

Fasting thermoneutral HP, HR and RT were monitored during the last week of the pre-exposure period and during days 27-30 and 48-50 of the 50 day differential temperature exposure period. Nineteen h prior to the monitoring of these parameters, the sheep, in groups of three, were transferred from their regular environments to an environmental chamber with an ambient temperature of 24-26°C. Thirty-two h prior to this and until the trial was over, feed but not water was withheld. HP was estimated by measuring respired gas VO2 and VCO2 using an open circuit respiratory pattern analyzer (Young *et al*.1975) connected to a ventillated face mask to which the sheep had become previously accustomed. A thermistor probe inserted 15 cm was used to monitor RT. Monitoring equipment was attached to the sheep prior to 1000 h and animals were left undisturbed for the balance of the trial. Commencing at 1100 h and continuing until 1530 h the respired gases of the three sheep were monitored over

alternating 30 min intervals. Heart rate and RT were recorded at 10 min intervals. During a 22 h period each week time spent standing by each sheep was recorded electronically to an accuracy of 0.5 min. This data will be reported elsewhere. Also time spent standing was recorded during the measurement of HP. Adjustments of HP, HR and RT for standing were made using covariate analysis (Steel and Torrie 1960).

Statistical analysis was by least squares analysis of variance and, where applicable, the Student-Newman-Keuls' test of mean differences (Steel and Torrie 1960).

**D. Results**

Table 1 presents mean plasma NA, A and DA values for the three treatment groups pre-exposure, during days 1-2 of exposure, and during days 9-44 of exposure. The mean plasma NA concentrations of the two cold treatment groups were significantly ($P<0.05$) greater than that of controls. The significant ($P<0.05$) treatment x period interaction for NA indicated that the plasma NA level of the two cold treatment groups but not of the control group increased during the differential temperature regimes. A maximum mean plasma NA level of 1.33 ng/ml was observed in the IC sheep during days 1-2. Temperature treatment also had a significant ($P<0.05$) effect on plasma A concentration but only the level of the CC sheep was greater ($P<0.05$) than that of controls. Although the intermittent cold treatment did cause a small increase in plasma A level, the significant ($P<0.05$) treatment by period interaction was mainly due to the substantial elevation in plasma A level of the chronic cold exposed group during days 1-2 and days 9-44. There was a significant ($P<0.05$) period effect on plasma DA level in that all groups had the lowest DA level during the pre-exposure period. The increase in plasma DA level upon the enforcement of the differential temperature regimes was greater in the two cold treatment groups than in the control group but this effect of treatment was not significant.

The only significant time of sampling effect was that found for the IC sheep during exposure days 1-2. During this

Table 1. Plasma catecholamine concentrations (ng/ml) during long term cold exposure (Mean ± SEM*)

| Temperature treatment | Exposure period | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pre-exposure | | | Days 1-2 | | | Days 9-44 | | |
| | NA‡ | A | DA | NA | A | DA | NA | A | DA |
| Control | 0.24 ±0.18 | 0.04 ±0.01 | 0.02 ±0.02 | 0.33 ±0.18 | 0.06 ±0.01 | 0.04 ±0.02 | 0.25 ±0.11 | 0.04 ±0.01 | 0.03 ±0.01 |
| Chronic cold | 0.25 ±0.17 | 0.06 ±0.01 | 0.05 ±0.02 | 0.74 ±0.18 | 0.15 ±0.01 | 0.11 ±0.02 | 0.86 ±0.11 | 0.13 ±0.01 | 0.10 ±0.01 |
| Intermittent cold | 0.26 ±0.18 | 0.04 ±0.01 | 0.05 ±0.02 | 1.33 ±0.17 | 0.08 ±0.01 | 0.13 ±0.02 | 0.78 ±0.12 | 0.07 ±0.01 | 0.09 ±0.01 |

Sources of variation

| | NA | A | DA |
|---|---|---|---|
| Treatment (T) | 0.05÷ | 0.05 | NS† |
| Period (P) | 0.01 | 0.01 | 0.05 |
| T X P | 0.05 | 0.05 | NS |

* Standard error of mean.

‡ NA=noradrenaline, A=adrenaline, DA=dopamine

÷ Level of significance ($P < .$).

† Nonsignificant ($P > 0.05$).

period the 2000 h mean level of NA (2.15 ng/ml) was significantly ($P<0.05$) greater than the 1100 h level of 0.51 ng/ml.

Mean blood hematocrit values are given in Table 2. Only the temperature treatment x period effect was significant ($P<0.01$). Hematocrit of CC sheep rose steadily with time of exposure while that of the control sheep declined somewhat and that of IC sheep remained relatively constant.

Mean plasma cortisol concentrations are given in Table 3. The large standard errors are a result of very high plasma concentrations of cortisol, greater than 20 ug/l, found in 12% of the samples. This variability is probably related to the intermittent nature of cortisol secretion from the adrenal cortex of sheep as shown by McNatty *et al.*(1972). The effect of temperature treatment on plasma cortisol concentration was not significant ($P>0.05$). Mean plasma cortisol concentration for days 9-44 was significantly ($P<0.001$) greater than during the previous two periods. This difference was mainly due to the increase in plasma cortisol concentration of the CC sheep during days 9-44, as indicated by the treatment x period interaction ($P<0.01$). Time of sampling did not influence plasma cortisol concentration but the time of sampling x period interaction approached significance ($P<0.06$). Thus during days 9-44 the mean 1100 h plasma cortisol concentration (19.0±2.0 ug/l) was higher than that found in 2000 h samples (8.3±2.0 ug/l) but such a noticeable diurnal trend was not apparent

Table 2. Blood hematocrit during long term cold exposure

| Temperature treatment | Mean ± SEM* Blood hematocrit (%) | | |
|---|---|---|---|
| | Exposure period | | |
| | Pre-exposure | Days 1-2 | Days 9-44 |
| Control | 25.7 ±0.5 | 24.8 ±0.5 | 23.6 ±0.3 |
| Chronic cold | 25.7 ±0.5 | 27.1 ±0.5 | 27.9 ±0.3 |
| Intermittent cold | 25.5 ±0.5 | 25.7 ±0.5 | 25.4 ±0.3 |
| Sources of variation | | | |
| Treatment (T) | NS† | | |
| Period (P) | NS | | |
| T X P | 0.01÷ | | |

* Standard error of mean.

† Nonsignificant (P>0.05).

÷ Level of significance (P<).

Table 3. Plasma cortisol response to long term cold exposure

| Temperature treatment | Mean ± SEM* Plasma cortisol (µg/l) | | |
|---|---|---|---|
| | Exposure period | | |
| | Pre-exposure | Days 1-2 | Days 9-44 |
| Control | 10.7 ±3.9 | 2.7 ±3.7 | 7.4 ±2.4 |
| Chronic cold | 3.9 ±3.7 | 4.8 ±3.9 | 22.0 ±2.4 |
| Intermittent cold | 3.7 ±3.9 | 7.9 ±3.7 | 11.6 ±2.6 |
| Sources of variation | | | |
| Treatment (T) | NS† | | |
| Period (P) | 0.001‡ | | |
| T x P | 0.01 | | |

* Standard error of mean.
† Nonsignificant (P>0.05).
‡ Level of significance (P<).

pre-exposure or during days 1-2 of exposure.

Mean values for cortisol entry rate and cMCR are given in Table 4. Only mean treatment values are given because the effect of period was not significant ($P>0.05$). The standard errors for cMCR are small but for cortisol entry rate are large, primarily because entry rate is calculated from plasma concentration of cortisol and there was large within animal and within treatment variation for plasma cortisol concentration.

The cMCR of the CC sheep was greater ($P<0.05$) than that of the control sheep but that of the IC sheep did not differ from that of the other two groups. A similar trend for cortisol entry rate was apparent but the differences between treatment groups were not significant ($P>0.05$).

Table 5 includes mean HP, HR and RT values for the three treatment groups measured before and twice during the differential temperature exposure period. The covariate coefficient for time spent standing was significant ($P<0.01$) only for HP. The HP means were adjusted to a mean standing time of 40% of total time which represented an elevation in HP of 0.026 $kJ/kg^{0.73}/min$ over the lying HP. Mean HP increased ($P<0.05$) from pre-exposure to days 27-30 and again ($P<0.05$) from days 27-30 to 48-50. The last increase was seen in control as well as cold-exposed sheep. There was no change in mean HR between the pre-exposure period and days 27-30 of exposure but mean HR measured during days 48-50 was greater ($P<0.05$) than that found during days 27-30. This

Table 4. Cortisol entry rate and metabolic clearance rate (cMCR) in control, chronic cold and intermittent cold exposed sheep

| | Control | Chronic cold | Intermittent cold |
|---|---|---|---|
| Entry rate (µg/min) | 10.0[a†] ±4.9* | 13.0[a] ±4.4 | 6.2[a] ±0.7 |
| cMCR (l/min) | 0.64[a] ±0.06 | 0.90[b] ±0.07 | 0.77[ab] ±0.09 |

* Standard error of mean.

† Means within a row with a common superscript were not significantly different (P>0.05).

Table 5. Mean fasting thermoneutral heat production, heart rate and rectal temperature of control (C), chronic cold (CC) and intermittent cold (IC) exposed sheep before and during the temperature treatment periods

| Temperature treatment | Period of exposure | | | | | | | | | Standard error |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-exposure | | | Days 27 - 30 | | | Days 48 - 50 | | | |
| | C | CC | IC | C | CC | IC | C | CC | IC | |
| Heat production ($kJ/kg^{0.73}/min$) | 0.222 | 0.220 | 0.204 | 0.221 | 0.248 | 0.238 | 0.253 | 0.281 | 0.264 | 0.009 |
| Heart rate (beats/min) | 64 | 56 | 60 | 54 | 62 | 53 | 75 | 72 | 59 | 4 |
| Rectal temperature ($^{o}C$) | 38.9 | 38.8 | 39.0 | 39.1 | 39.0 | 38.9 | 39.1 | 38.9 | 38.8 | 0.1 |

| Sources of variation | Heat production | Heart rate | Rectal temperature |
|---|---|---|---|
| Treatment (T) | NS† | NS | NS |
| Period (P) | 0.001÷ | 0.025 | NS |
| T X P | NS | NS | NS |

† Nonsignificant ($P>0.05$).

÷ Level of significance ($P<$).

latter change in HR was mainly due to an increase in HR of the contol sheep. Rectal temperature was not influenced by treatment or period of exposure.

## E. Discussion

Pre-exposure plasma NA concentrations determined using a radioenzymatic assay were similar to the thermoneutral plasma NA levels in sheep of 0.24 ng/ml and 0.31 ng/ml found by Thompson *et al.*(1978) and Christopherson *et al.*(1978), respectively. In the latter studies plasma catecholamine levels were determined using a fluorometric method. The plasma NA level of the sheep exposed to chronic cold was about twice the level of 0.43 ng/ml found by Christopherson *et al.*(1978) in sheep exposed four weeks to 2-5 °C. The sheep in the present experiment were smaller and were sheared more frequently and therefore may have been more cold stressed than those in the experiment of Christopherson *et al.*(1978). Webster *et al.*(1969a) found that sheep urinary level of NA was proportional to the degree of chronic cold stress. This may also be true with respect to plasma NA level. Leduc (1961) and Shum *et al.* (1969) have shown that the laboratory rat urinary level of NA is very high during the first few weeks of moderate cold exposure but then declines substantially-thereafter as the exposure becomes chronic. Such a time trend was not observed in the plasma NA levels of CC sheep in which elevated NA levels persisted for the duration of the cold exposure.

Blood sampling of the IC sheep was always done during the thermoneutral phase of the diurnal temperature cycle. The reason for the very high 2000 h plasma NA level of these sheep during days 1-2 is unknown. The mean 2000 h value of

2.15 ng/ml is higher than the highest value of 1.20 ng/ml observed by Thompson *et al.*(1978) in sheared sheep acutely exposed to -20°C. The data seem to suggest that the IC sheep, after only one or two nightly cold exposures, developed a daily plasma NA rhythm. Increases in muscular activity results in high circulating levels of NA in man (Galbo *et al.*1977), but a wide diurnal variation in degree of activity of the IC sheep was not observed. That the diurnal fluctuation in plasma NA level did not persist into days 9-44 indicates that the sheep became habituated to the intermittent cold treatment. Habituation has been defined by Bligh and Johnson (1973) as a decrease in response to or detection of repeated stimulation and in this case probably involved a stabilization in rate of secretion of NA from sympathetic nerve endings and/or the adrenal medulla with prolonged intermittent cold exposure.

Pre-exposure plasma A concentrations were slightly lower than the thermoneutral plasma A concentrations in sheep reported by Thompson *et al.*(1978) and Christopherson *et al.*(1978). The greater than two-fold increase in plasma A upon chronic cold exposure was similar to that found by Christopherson *et al.*(1978) for sheep exposed four weeks to 2-5 °C. Thus the adrenal medullary response to chronic cold was similar in the two experiments and the present data indicates that the response of the sheep adrenal medulla to chronic cold exposure changes very little over a period of 44 days. A comparable sustained elevation in adrenal

adrenaline output as reflected by urinary A levels of rats exposed to 4°C for four weeks was reported by Shum *et al.*(1969) but Leduc (1961) observed an initial five fold increase in urinary A level followed by a decline to near pre-exposure levels in rats exposed to 5°C for four weeks. Adrenaline secreted from the adrenal medulla may be involved in the metabolic acclimation of sheep to chronic cold exposure. Tanche (1976) has shown that daily adrenaline injections in thyroidectomized and adrenal demedullated dogs result in a cellular sensitization to the calorigenic effect of adrenaline. It is possible that the elevated plasma adrenaline levels associated with prolonged cold exposure induces a similar type of sensitization in sheep. Like daily injections of A in the dog, daily injections of NA have been found to sensitize rats to the calorigenic effects of NA (Leblanc and Pouliot 1964). Since both the CC and IC sheep of the present experiment showed persistent elevation in plasma NA levels this might also contribute to the process of metabolic acclimation. Graham (1977) showed that the hyperglycemic response to A infusion is potentiated in sheep by chronic cold exposure and we have evidence (Study 2) that the same is true for the calorigenic effect of A in sheep.

The level of plasma A in the IC sheep was only slightly greater than that of the control sheep but it was very likely that the IC sheep had elevated plasma A levels during the cooler phase of the diurnal temperature cycle and therefore may also have developed sensitization to the

metabolic effects of adrenaline.

Pre-exposure plasma DA levels were slightly lower than those found previously in sheep and goats (Kelly *et al.* 1970) and in resting undisturbed man, cat and cow (Buhler *et al.*1978). The absence of a significant effect of temperature treatment on plasma DA level supports the observation of Buhler *et al.*(1978) that the increase in plasma DA concentration under stressful conditions is less than the increase in A or NA.

Elevated hematocrit during chronic cold exposure, like that seen in sheep acutely exposed to severe cold (Thompson *et al.*1978), is probably a result of splenic contraction. In the IC sheep hematocrit was probably not elevated because blood samples were taken three and eleven h into the warmer phase of the temperature cycle and Thompson *et al.*(1978) have shown a return to pre-exposure hematocrit by two h following acute cold exposure of sheep.

The plasma concentrations of glucocorticoids in man (Weitzman 1971), marsupials (McDonald and Bradshaw 1977) and sheep (McNatty *et al.*1972) fluctuates considerably due to the episodic release of glucocorticoids from the adrenal cortex. Frequency of bursts of adrenal cortex activity therefore determines plasma glucocorticoid concentration (Akerstedt and Levi 1978). McNatty *et al.* (1972) have calculated that the adrenal cortex of a relaxed sheep releases essentially no cortisol two thirds of the time. They also observed that within an individual sheep, plasma

concentration of cortisol fluctuated within the range of 0 to 10 ug/l for most of the day but a few peak concentations greater than 20 ug/l occured between 800 h and 1400 h. The present data are in agreement with that of McNatty *et al.*(1972) in that control sheep plasma cortisol concentration averaged 6.9 ug/l; a value which includes some very high concentrations that occured most often during the morning sampling period. Such large variations in plasma cortisol concentration makes it difficult to detect relatively small treatment effects. Despite this, it was found that CC treatment elevated mean plasma cortisol concentration. This effect was probably not a reflection of differences in nutritional status because all the sheep were fed at a maintenance level. Acute cold exposure of sheep results in elevated plasma cortisol concentration (Panaretto and Vickery 1972). The nightly cold exposure of the IC sheep similarily would be expected to promote cortisol secretion but evidence of such stimulation was not present at the time of blood sampling during the warmer phase of the diurnal temperature cycle. It is probable that the plasma cortisol response to nightly acute cold exposures is short lived as is the response to adrenocorticotropin injection in sheep (Beaven *et al.*1964).

The control sheep mean±SEM cortisol entry rate of 10.0±4.9 ug/min is similar to that recorded previously by Panaretto and Vickery (1970) (7.7±3.3 ug/min) and Panaretto and Vickery (1972) (6.9±3.3 ug/min) for sheep in a

thermoneutral environment. Panaretto and Vickery (1972) found cortisol entry rate increased to 19.2±15.3 ug/min upon acute cold exposure. In neither the present study nor that of Panaretto and Vickery (1972) did the effect of cold seem statistically significant. This was mainly because of the high variability in plasma cortisol concentration. That the cortisol entry rate of the IC sheep (6.2±0.7 ug/min) was somewhat lower than that of the control sheep may indicate that the IC sheep reduced cortisol secretion substantially when the nightly cold stress period ended. Christison and Johnson (1972) found that cortisol turnover rate of cattle can be depressed by chronic exposure to heat. It would be of interest to monitor the cortisol entry rate of intermittent cold exposed sheep over the entire 24 h cycle to determine if elevated cortisol entry occurs during the cooler phase and depressed cortisol entry occurs during the warmer phase of the diurnal temperature cycle. If so, overall changes in metabolism due to cortisol might be negligible in that target tissues would receive daily cortisol input of a magnitude similar to that of non-cold exposed animals.

The magnitude of the elevation in cMCR due to chronic cold exposure of sheep in the present experiment is similar to that found by Panaretto (1974) where the cMCR of sheep increased from 0.86 l/min to 1.03 l/min upon exposure to 3°C and wetting. Panaretto (1974) also showed that splanchnic metabolism (primarily hepatic) was responsible for 90-100% of cortisol clearance in sheep and that the increase in cMCR

during acute cold exposure was a result of an increase in hepatic blood flow. Greenway and Lawson (1966) have shown that A infusion in the anaethesized cat can cause increased portal vein blood flow. In the present experiment, plasma A level was significantly elevated in the CC but not in the IC sheep and this may explain why cMCR was elevated significantly in the CC but not the IC sheep.

In summary, it appears that the CC sheep exhibited signs of elevated cortisol metabolism and this was to some degree reflected by an elevated plasma cortisol concentration especially during the morning sampling periods of days 9-44. Elevated cortisol metabolism could contribute to an increased glucose flux which has been shown to occur in cold-acclimated sheep (McKay *et al*.1974). The IC sheep showed no evidence of increased cortisol metabolism during the thermoneutral phases of the diurnal temperature cycle.

The 13 to 17% increase in HP for sheep fasted 51 h after 27-30 days of exposure to the two cold temperature regimes is comparable to that resulting from prolonged exposure of sheep to a cold Albertan winter environment or a cold indoor environment (Webster *et al*.1969b). In the present experiment the HP of both the control and cold exposed groups increased further between the 27-30 d and 48-50 d measurement periods. During the 48-50 d measurement period the control, but not cold exposed, sheep exhibited signs of emotional discomfort; head and foreleg movement. This increased activity may have been the cause of the high

HP and HR of the control sheep at this time. The increase in resting HP of the IC as well as CC sheep indicates the development of metabolic acclimation to cold in both treatment groups. In this respect metabolic acclimation resembles the acclimatization of sheep to winter conditions (Webster *et al.*1969b). Slee (1972) found that chronic exposure of sheep to four daily one h periods below 0°C. did not cause an increase in thermoneutral heart rate as did chronic cold exposure and concluded, on the basis of heart rate measurements, that repeated short cold shocks do not induce metabolic acclimation as characterized by increased resting HP. In the present experiment the elevation in resting HP brought on by intermittent cold exposure was not accompanied by an increase in HR. An elevation in resting HP in the absence of an elevation in resting HR may also have existed in the short cold shock habituated sheep in the study of Slee (1972).

The present study indicates that both chronic constant and intermittent cold exposure of sheep result in the development of metabolic acclimation as reflected in increased resting HP and persistent elevations in plasma NA concentrations, presumably reflecting increased sympathetic activity.

Elevation in cMCR during chronic cold exposure is probably accompanied by an elevation in cortisol entry rate. However, cortisol entry rate was calculated from the plasma cortisol concentration which varied widely and caused the

derived entry rate values to also be highly variable. Cortisol metabolic clearance rate and probably entry rate are not elevated during thermoneutral daytime periods following nightly moderate cold exposures.

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## III. Study 2

# Effects of Adrenaline and Noradrenaline on the Heat Production and Rumen Motility of Warm- and Cold-acclimated Sheep

### A. Abstract

The heat production (HP), heart rate (HR), respiration rate (RR), rumen motility, and body temperature responses to adrenaline (A) and noradrenaline (NA) intrajugular infusions at 0.00, 0.15, 0.30, 0.60, and 0.90 ug/kg/min were studied in ten shorn wethers which had been chronically (3-8 weeks) exposed to warm (19-24°C) or moderately cold (8-13°C) temperatures. Heat production, as estimated from respired gas analysis, increased 40-45% with all doses of A and the effect was potentiated by chronic cold exposure. Only the higher dose rates of NA induced an increase in HP. The maximum HP increase due to NA was 30% and the effect was not influenced by chronic cold exposure. Thermoneutral HP was greater by 18-19% in cold-acclimated as compared to warm-acclimated sheep. Corresponding to the HP effects of A and NA, all doses of A and the highest dose of NA resulted in slight increases in rectal temperature. RR increased with increased dose rate of NA but only the highest dose of A resulted in an increase in RR. HR, rectal temperature and RR responses to A and NA were not influenced by cold-acclimation. The initial response of the rumen to A was an increase in motility but after one hour of infusion an

inhibition of motility was seen, especially in the warm-acclimated sheep. NA also inhibited rumen motility to a greater extent in the warm-acclimated sheep.

## B. Introduction

Acclimation of rodents to low environmental temperatures is associated with hypertrophy of brown adipose tissue (Cameron and Smith 1964) and morphological changes in skeletal muscle mitochondria (Behrens and Himms-Hagen 1977). Both developements (Foster and Frydman 1978) appear to be responsible for the cold acclimation characteristic of increased nonshivering thermogenic effects of noradrenaline (NA) (Hseih *et al*.1957; Depocas 1960; Jansky 1971) and adrenaline (A) (Himms-Hagan 1969). An enhanced nonshivering thermogenic response to catecholamines is believed to be the mechanism by which cold-acclimated rodents have increased ability to withstand cold temperatures (Sellers and You 1950; Jansky 1966). Adult sheep and cattle also show metabolic acclimation to low environmental temperatures. Acclimation is characterized by an elevated thermoneutral heat production (HP) (Webster *et al*.1969a; Young 1975), an enhanced ability to raise HP during very severe cold exposures (Webster *et al*.1969a), and possibly a replacement of shivering thermogenesis with nonshivering thermogenesis (Slee 1972; Young 1975). Although very young ruminants show a calorigenic response to catecholamines, this response, as well as brown adipose tissue, appear to be absent by one month after birth (Thompson and Jenkinson 1969; Alexander *et al*.1975). Research by Webster *et al*.(1969b) has led to the belief (Heldmaier 1971; Webster 1974) that the calorigenic effect of catecholamines in ruminants cannot be

reestablished later in life by cold-acclimation. Webster *et al.*(1969b) found that 30 minute infusions of A or NA at the high dose rate of 1.0 ug/Kg/min were not significantly calorigenic in either warm- or cold-acclimated sheep. However, lower doses of catecholamines have been found to be calorigenic in humans (Joy 1963; Budd and Warhaft 1966; Kang *et al.*1970) and in rodents very high doses of catecholamines are sometimes less calorigenic than lower doses (Himms-Hagen *et al.*1972; Bartunkova *et al.*1971).

The present experiments were designed to determine if doses of A and NA less than 1.0 ug/Kg/min were calorigenic in warm- and cold-acclimated sheep. Additional parameters such as heart rate (HR), respiration rate (RR), rectal temperature (RT) and rumen motility were monitored during catecholamine infusions because these factors are also influenced by acclimation of sheep to cold (Sykes and Slee 1968, 1969; Westra and Christopherson 1976).

## C. Material and Methods

### Experiment I - Adrenaline Infusion

Ten rumen-fistulated eight-month-old wethers were used. The sheep were held in individual stalls for eight weeks in a 19-24°C holding room or an environmental chamber at an initial ambient temperature of 13°C. All sheep were sheared to a fleece depth of 0.5 cm at the beginning of the trial and during week 3. In an effort to offset any decrease in heat loss due to fleece growth, the environmental chamber temperature was reduced 1°C weekly following the first shearing and was raised to 13°C and was then reduced 1°C weekly following the second shearing. Good quality alfalfa pellets were fed twice daily at 0800 h and 1700 h. Each meal consisted of 700 g and 1100 g for the sheep in the warm and cold environments, respectively. The differential feeding rate was intended to prevent major differences in the body fat stores of the two treatment groups. To this end, a mean±SEM body weight of 51.0±0.5 Kg was maintained by both treatment groups throughout the experiment. Water and cobalt iodized salt were available at all times and room lighting was continuous.

The metabolic responses to A and saline infusions were studied during weeks four to eight. Doses were administered following the design of a pair of Latin squares as shown in Table 6. Infusion trials were carried out on pairs of sheep in an environmental chamber at ambient temperatures ranging from 20°C to 23°C. At approximately 1630 h the evening prior

Table 6. Time schedule and dose rates for intravenous adrenaline infusions in warm- and cold-acclimated sheep

| | Group | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Warm-acclimated | | | | | | Cold-acclimated | | | | |
| | | Week | | | | | | Week | | | |
| Weekday | Animal | 4 | 5 | 6 | 7 | 8 | Animal | 4 | 5 | 6 | 7 | 8 |
| Monday | 1 | 0.15* | 0.30 | 0.60 | 0.90 | 0.00 | 6 | 0.15 | 0.90 | 0.00+ | 0.00 | 0.30 |
| Tuesday | 2 | 0.30 | 0.60 | 0.15 | 0.00 | 0.90 | 7 | 0.00 | 0.60 | 0.30 | 0.90 | 0.15 |
| Wednesday | 3 | 0.00 | 0.15 | 0.90 | 0.60 | 0.30 | 8 | 0.30 | 0.00 | 0.90 | 0.15 | 0.60 |
| Thursday | 4 | 0.60 | 0.90 | 0.00 | 0.30 | 0.15 | 9 | 0.60 | 0.15 | 0.00 | 0.30 | 0.90** |
| Friday | 5 | 0.90 | 0.00 | 0.30 | 0.15 | 0.60 | 10 | 0.90 | 0.30 | 0.15 | 0.60 | 0.00 |

*Dose of adrenaline (µg/kg/min).

+Incorrect dose administered.

**Trial not executed.

to an infusion trial the sheep were transferred to individual stalls in the thermoneutral chamber and feed but not water was witheld until after the infusion trial. Also at the time of transfer a polyvinyl catheter (1.8 mm O.D.) of 3 m length was inserted 15 cm into a jugular vein of each sheep through a 13 guage needle. Prior to 1000 h on the day of an infusion trial the monitoring apparatus was attached to the sheep and recording cables plus infusion catheters were extended through portholes in the chamber wall. The sheep were then left undisturbed until the end of the infusion trial. Infusions commenced at 1200 h and were of 2.5 h duration.

Adrenaline bitartrate (Sigma) or saline infusates were prepared with sterile 0.9% saline containing 0.1 g/l ascorbic acid as an antioxidant. The solutions were prepared immediately prior to the infusion period and were kept in an ice-water bath at all times. A Technicon Autoanalyzer II proportioning pump III was employed to maintain an infusion volume rate of 0.60 ml/min.

Physiological parameters were monitored for one hour pre-infusion, during infusion, and for one hour post-infusion. HP ($kJ/kg^{0.73}/min$) was estimated from measurements of respired gas $VO_2$ and $VCO_2$ determined with an open circuit respiratory pattern analyzer as described by Young *et al.*(1975). During weeks one to three the sheep were pre-conditioned to wearing the face masks used to collect respired air. A thermocouple probe inserted 15 cm was used

to monitor RT and a water filled balloon with tygon tubing extension to a pressure transducer was used to monitor rumen motility. Rumen balloon pressure and HR were recorded with a Sanborn physiograph. A kymograph trace of RR was obtained by the use of an air filled tubing attached to a flexible hose extended around the abdomen of the sheep. A flexible cord attached to the back of the sheep caused the interruption of a microswitch circuit when the sheep moved to a lying position. An event recorder was used to record, to an accuracy of 0.5 min, time spent standing or lying. Throughout an infusion trial HR and RT were recorded every ten minutes for both sheep. RR, rumen motility, and standing/lying activity were monitored continuously for both sheep and the respired gas analyzer was alternated from one sheep to the other every ten minutes. When the respiratory pattern of a particular sheep was not being analyzed the mask of that sheep was ventilated with an auxillary gas pump.

**Experiment II - Noradrenaline Infusion**

Ten rumen fistulated 14 month old wethers, six of which had, 5 months previously, been used in Experiment I, were used. The sheep were held in individual stalls for eight weeks in a 21-24°C holding room or an environmental chamber at an initial ambient temperature of 8-9°C. The feeding and management regime was identical to that of Experiment I and sheep maintained a mean±SEM body weight of 47.0±0.3 kg throughout the experiment. But for a difference in dose

randomization the administration of noradrenaline bitartrate (Sigma) during weeks four to eight was as indicated for A infusions in Table 6. In error the warm-acclimated sheep that should have received NA infused at the rate of 0.90 ug/kg/min during week five received the 0.00 ug/kg/min dose rate. At approximately 1500 h the evening prior to an infusion trial the sheep were transferred to individual stalls in a thermoneutral (22-25°C) chamber and feed but not water was witheld until after the infusion trial. Prior to 0900 h on the day of an infusion trial a polyethylene infusion catheter (0.96 mm O.D.) of 3 m length was inserted 15 cm into a jugular vein of each sheep through a 16 guage needle. In addition to the monitoring apparatus described for Experiment I, thermocouples were used to monitor ear and trunk skin temperatures. The thermocouples were taped to shaved areas of skin on the left ear dorsal surface 1.5 cm lateral to the midline and 6 cm distal to the ear base and on a trunk site 10 cm anterior to the tail and 8 cm lateral to the spine. A 24 channel temperature recorder (Honeywell Electronic-19) was used to record temperatures at 0.3 minute intervals. By 0930 h all monitoring apparatus had been attached to the sheep and the animals were left undisturbed until the end of the infusion trial. The infusions commenced at 1130 h and were 2.5 h in duration.

Statistical analysis of the data of Experiments I and II consisted of separate least squares analyses of variance for unequal numbers (Steel and Torrie 1960) where variables

included were acclimation temperature, dose, week, time of infusion, and all meaningful interactions of these main effects. For the statistical analysis, time of infusion was divided into five intervals; pre-infusion, infusion hour 1, infusion hour 2, last 0.5 hour of infusion and post-infusion. Time spent standing as a percentage of total time in a given interval was included as a covariate. Differences between means were compared using the Student-Newman-Keuls' test (Steel and Torrie 1960).

## D. Results

All mean values presented in Figures 1-3 and Table 7 have been adjusted by covariate analysis to mean standing times of 27% and 31% of total time for Experiments I and II, respectively.The covariate was significant ($P<0.025$) for HP, RT, and RR in Experiment I and was significant ($P<0.001$) for HP and HR in Experiment II. In Experiment I standing 27% of the time was associated with an elevation in HP, RT, and RR of 0.01 $kJ/kg^{0.73}/min$, 0.16°C, and 0.4 respirations/min, respectively, over the lying values. In Experiment II standing 31% of the time was associated with an elevation in HP and HR of 0.02 $kJ/kg^{0.73}/min$ and 5.5 beats/min, respectively, over the lying values.

### Experiment I - Adrenaline Infusion

The histogram of Figure 1A represents the mean HP values during A infusion trials. All four doses of A were equally potent ($P<0.001$) in increasing HP. The mean HP during the last half hour of infusion was 43% greater than that found pre-infusion or during saline infusion. Figure 2 illustrates the significant ($P<0.01$) effect of temperature of acclimation on the HP response to the various infusion rates of A. During saline infusion the mean HP of the cold-acclimated (CA) sheep was 18% greater than the mean HP of the warm-acclimated (WA) sheep and the HP difference between treatment groups increased with the increase in dose rate. Thus, in reference to Figure 1A, the increase in HP caused by A was greater than 43% in the CA sheep and less

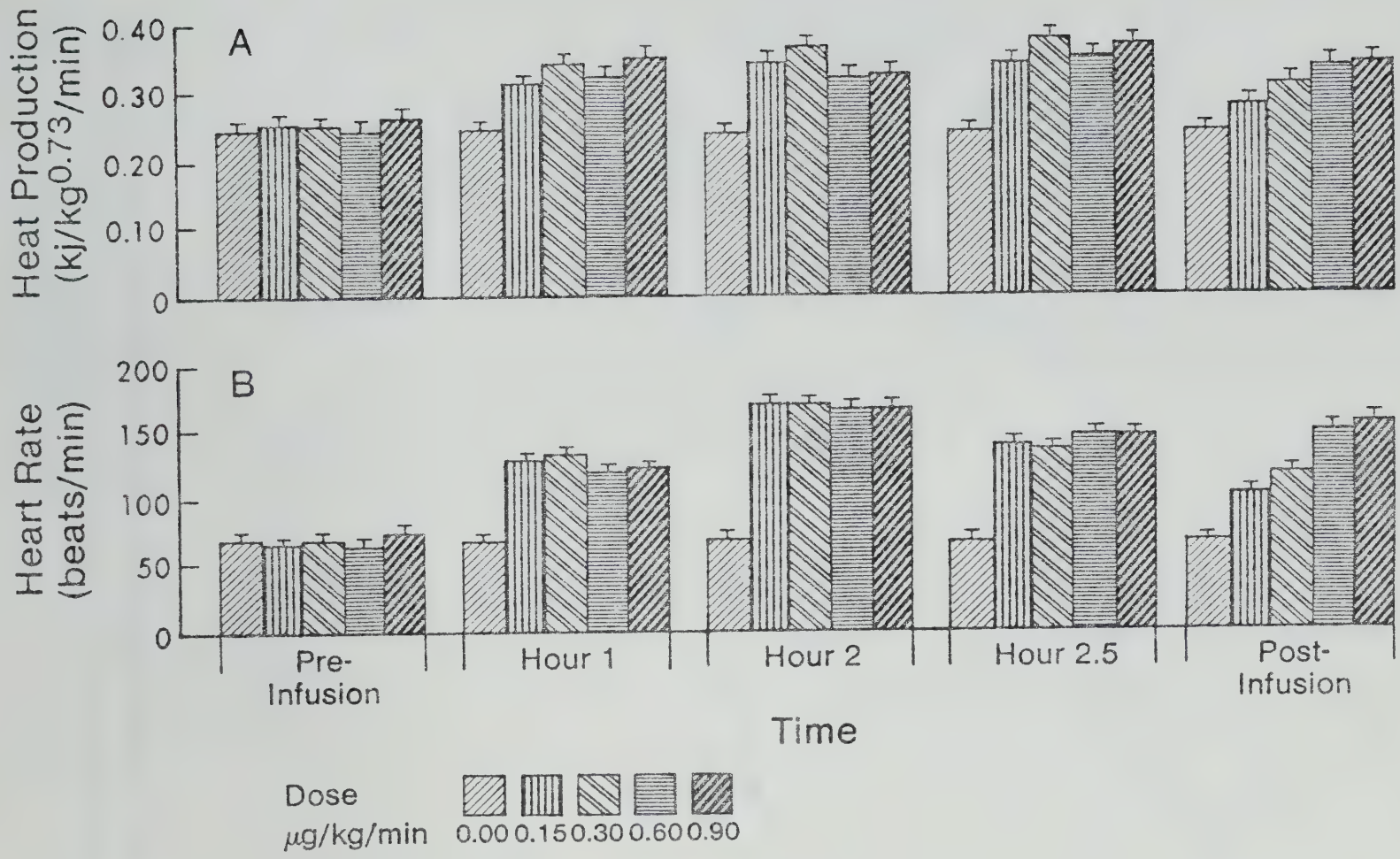


Figure 1. Effects of various doses of infused adrenaline on the heat production (A) and heart rate (B) of sheep (Mean ± SEM).

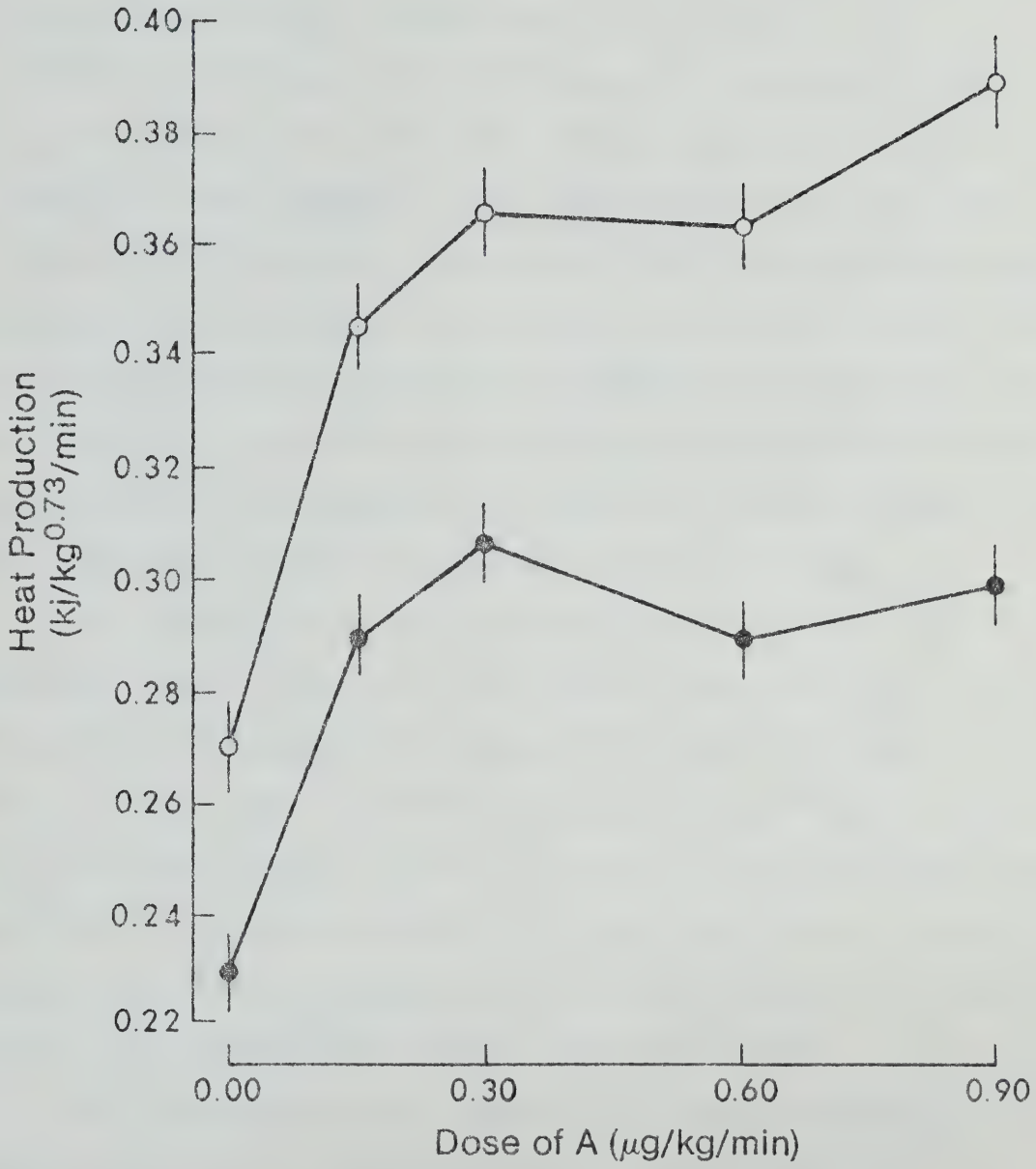


Figure 2. Mean ± SEM heat production of warm-acclimated (-●-) and cold-acclimated (-o-) sheep with various doses of infused adrenaline (A).

than 43% in the WA sheep. The maximum HP response to A in the WA sheep was achieved with a dose of 0.30 ug/kg/min but in CA sheep increasing the dose of A from 0.30 ug/kg/min to 0.90 ug/kg/min had an additional effect on HP. As illustrated in Figure 1B, all doses of A resulted in a 2.5 fold increase in HR ($P<0.001$). HR was maximum during the second hour but tended to fall during the last half hour of A infusion. The amount by which HP and HR declined during the post-infusion period was inversely related to dose rate. The HR response to A was not influenced by temperature of acclimation. The overall mean±SEM HR of 127±2 beats/min of the CA sheep was slightly but not significantly greater than the mean±SEM of 107±2 beats/min for the WA sheep.

The mean±SEM RT of the CA sheep (39.8±0.1°C) was greater ($P<0.01$) than that of the WA sheep (39.3±0.1°C). RT tended to increase with increase in dose of A, especially in the WA sheep, but in neither group was the effect of dose of A on RT significant ($P>0.05$). During saline infusions the RR of the CA sheep was greater ($P<0.05$) than that of the WA sheep and in both groups the two highest doses of A caused a small but significant ($P<0.05$) increase in RR.

The rumen motility response to A was difficult to quantify in that A appeared to influence frequency, amplitude, and rythmicity of rumen contractions. In 41% of the A infusion trials a temporary increase in frequency of rumen contractions was observed during the first hour of infusion. In 90% of the WA and 59% of the CA sheep A

infusion trials a depression in frequency of rumen contractions was observed after the first hour of infusion. On these occasions contraction amplitude was also often depressed and regular strong contractions were often completely absent and a pattern of very low amplitude very frequent increases in pressure was instead recorded. The greater inhibitory effect of A on rumen motility of the WA as compared to the CA sheep was particularly evident at the lowest infusion rates.

**Experiment II - Noradrenaline Infusion**

HP decreased with time during saline infusion or NA infusion at the rate of 0.15 ug/kg/min, but increased with time of infusion when NA was infused at the three highest dose rates (Figure 3A). This interaction between time of infusion and infusion dose rate was significant ($P<0.01$). The largest HP response to NA infusion was the 30% elevation in HP observed during the last half hour of NA infusion at 0.90 ug/kg/min. Increases in HP due to the 0.60 and 0.30 ug/kg/min infusions of NA were about 18% and 8%, respectively, when compared to pre-infusion HP. There was no effect of temperature of acclimation on the HP response to NA infusion but the overall mean HP of the CA sheep (0.31 $kJ/kg^{0.73}/min$) was greater ($P<0.001$) than the overall mean HP (0.26 $kJ/kg^{0.73}/min$) of the WA sheep. Figure 3B shows that the HR response to NA infusion increased with dose rate. Only the dose rates of 0.60 ug/kg/min and 0.90 ug/kg/min increased HR ($P<0.05$) above the values found with

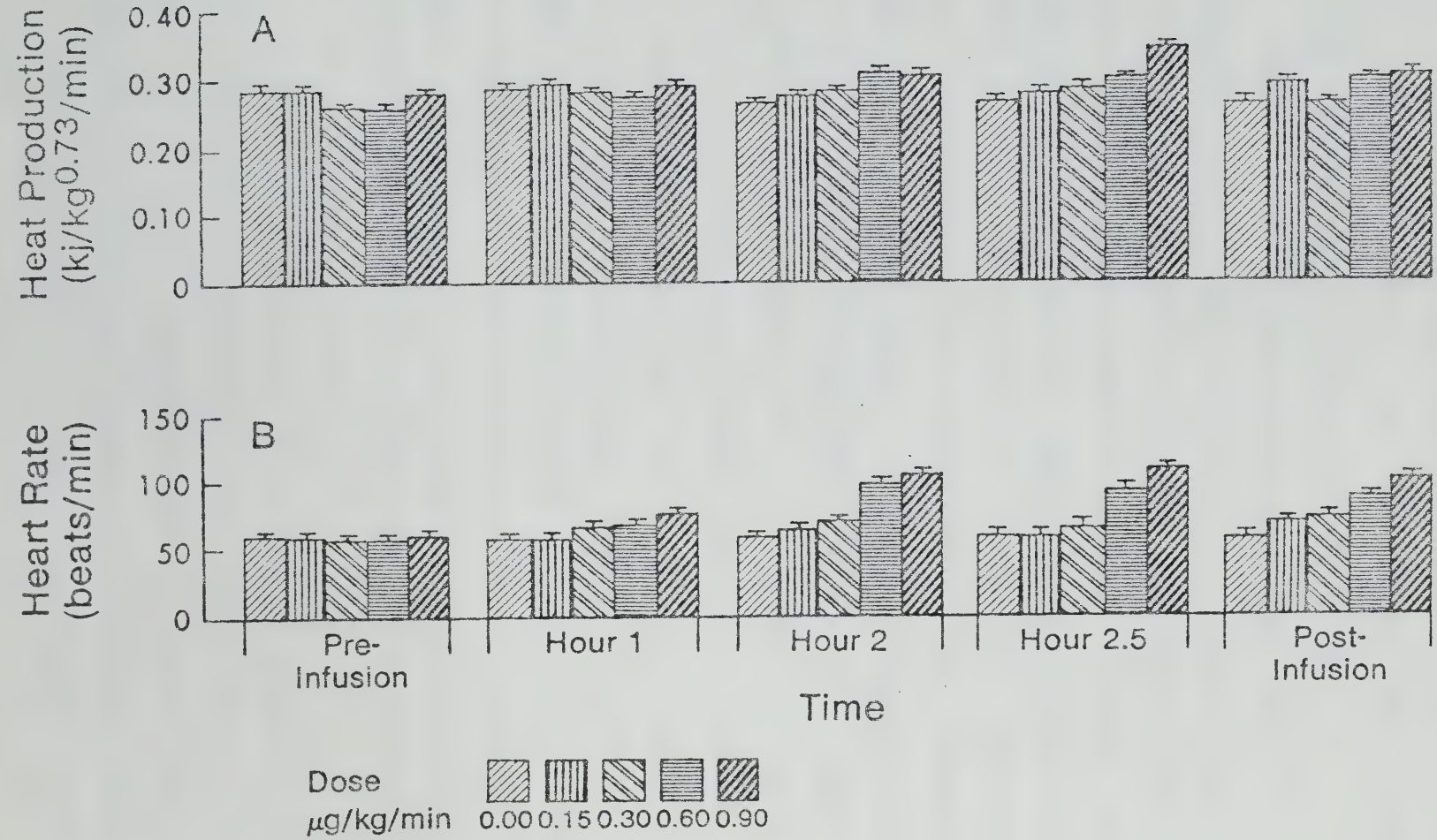


Figure 3. Effect of various doses of infused noradrenaline on the heat production (A) and heart rate (B) of sheep (Mean ± SEM).

saline infusion. Also, the mean HR for these two highest dose rates differed significantly ($P<0.05$). The mean HR of the WA sheep was usually lower than that of the CA sheep but this difference was not significant ($P>0.05$).

Table 7 contains the mean±SEM RT,RR, and skin temperature values for the various NA infusion trials. RT increased ($P<0.05$) only with the highest dose of NA. All doses of NA resulted in mean RR greater ($P<0.05$) than that found for saline infusion. Temperature of acclimation had no effect on RT or RR. Vasodilation of ear skin, as indicated by an increase in ear skin temperature (EST), tended to occur with the lower doses of NA but the highest dose of NA had no such effect. The mean±SEM EST of the CA sheep (31.4±0.3°C) was greater ($P<0.05$) than that of the WA sheep (24.7±0.3°C). There was a trend towards an increase in trunk skin temperature (TST) with time during NA infusions but this effect was not significant ($P>0.05$). The TST values shown in Table 7 reflect both differences in pre-infusion skin temperatures for the different dose rates and the slight increase in TST that tended to occur during NA infusion. Temperature of acclimation had no effect on the EST or TST responses to NA.

There was no noticable effect of NA infusion on the rumen motility of the CA sheep but during half of the NA infusions at the two highest dose rates the WA sheep rumen contraction frequency was observed to decrease and during a few trials regular motility ceased completely. On these

Table 7. Rectal temperature (RT), respiration rate (RR), ear skin temperature (EST), and trunk skin temperature (TST) of sheep during infusion of various doses of noradrenaline

| Dose µg/kg/min | RT (°C) | RR (resp./min) | EST (°C) | TST (°C) |
|---|---|---|---|---|
| 0.00 | 38.4±0.1[a+‡] | 11.5±0.6[a] | 27.6±0.5[ab] | 32.6±0.2[a] |
| 0.15 | 38.5±0.1[a] | 13.2±0.6[b] | 28.4±0.5[ab] | 33.1±0.2[ab] |
| 0.30 | 38.5±0.1[a] | 13.8±0.6[bc] | 29.3±0.5[a] | 33.3±0.2[bc] |
| 0.60 | 38.4±0.1[a] | 14.8±0.6[bc] | 28.1±0.5[ab] | 32.9±0.2[ab] |
| 0.90 | 38.8±0.1[b] | 15.8±0.7[c] | 27.0±0.5[b] | 33.7±0.2[c] |

[+] Values represent means ±SEM for nine or ten sheep.

[‡] Means within a column with similar superscripts were not significantly different ($P>0.05$).

occasions there appeared a pattern of very low amplitude very frequent increases in rumen pressure.

## E. Discussion

Metabolic acclimation to cold was evident in Experiment I from the 18% greater thermoneutral HP during saline infusion of the CA as compared to the WA sheep and the slightly greater overall mean HR of the CA sheep. In Experiment II overall mean HP of the CA sheep was 19% greater than that of the WA sheep; a difference which was not due to treatment differences in response to NA infusion. It can be calculated from Kleiber's (1974) estimate of the heat increment of hay that the calorigenic effect of the extra 800g of feed consumed by the cold exposed sheep would increase HP by about 0.025 kJ/Kg$^{0.73}$/min. Since the sheep were fasted 20 h prior to HP measurements, a heat increment effect due to the difference in feeding level would be considerably less than the HP difference found between WA and CA sheep.

The calorigenic response to A in Experiment I was much greater than any calorigenic response to A or NA previously reported for intact post-neonatal large mammals (Joy 1963; Steinberg *et al.*1964; Budd and Warhaft 1966; Thompson and Jenkinson 1969; Webster *et al.*1969b; Kang *et al.*1970; Alexander *et al.*1975; Tanche 1976). The present adjustment of HP values for standing/lying activity by covariate analysis significantly reduced variation which might otherwise have partially masked treatment effects on HP including the calorigenic effects of A and NA. Bennett (1972) has shown a significant relationship between time

spent standing and the HP of sheep. Webster *et al.*(1969b) found a slight (5%) increase in HP of WA and CA sheep upon 0.5 h, 1.0 *u*g/Kg/min A infusions. Standing/lying activity was not reported and may have introduced variation into the HP results. Webster *et al.*(1969b) did report a decline in HP of the WA sheep during saline infusion trials which might indicate that the sheep were more active prior to than during infusions. Such a time and possibly stress related trend in resting HP could have masked much of the HP response to A infusion. That HP did not decline during saline trials of Experiment I indicates that the animals were calm throughout. The calorigenic response to the highest rate of A infusion was, at least in the WA sheep, no greater than the response to the lowest rate of infusion (0.15 *u*g/Kg/min). The lowest infusion rate is similar to the quantity of A that can be released by the adrenal glands of adult sheep (Comline and Silver 1961) and cattle (Silver 1960) under conditions of splanchnic nerve stimulation. Since A reaches its target tissues via the circulation, intravenous infusion should mimic the effects of endogenous release. Thus A appears to be calorigenic in sheep at physiological dose rates.

In Experiment II the slight decline in HP of the sheep during saline and the lowest dose of infused NA probably indicates that the sheep were not completely calm during the initial phase of the infusion trials. Covariate analysis with respect to time spent standing would not have adjusted

HP values for time related changes in head movement, leg movement, or the HP associated with frequent movement from the lying to the standing position. The difference in HP between the saline and NA infused sheep was most evident when NA was given at the two highest dose rates. Very high intravascular dose rates of NA are often required to produce physiological responses because the level of NA at the sympathetic nerve terminal-effector cell junction is much higher than the plasma level (Neil 1975). The use of higher dose rates than those used in the present experiment might have resulted in a greater calorigenic response but this seems unlikly since the increases in HP with the two highest dose rates of NA were similar during the first two hours of infusion. From the relationship shown by Heldmaier (1971) between body weight and the dose rate of NA that will cause maximum calorigenesis, a dose rate of 0.4 *u*g/kg/min would be expected to cause the maximum HP response in the sheep of the present experiment. Steinberg *et al.*(1964) found a 21% increase in oxygen consumption of adult humans when NA was infused at a rate of 0.2-0.4 *u*g/kg/min. Lower doses have usually resulted in a less than 14% increase in the HP of humans (Reale *et al.*1950); even when the subjects were cold-acclimated (Joy 1963; Budd and Warhaft 1966; Kang *et al.*1970). Smaller mammals such as the monkey, cat, and dog, respectivly, have shown 20%, 36%, and 50% increases in oxygen consumption upon NA infusion or injection (Hemingway *et al.*1964; Nagasaka and Carlson 1965; Chaffee and Allen

1973). The response of the monkey (Chaffee and Allen 1973) but not the dog (Nagasaka and Carlson 1965) increased following cold-acclimation. Webster *et al.*(1969b) found no increase in the HP of WA and CA sheep during half hour 1.0 ug/kg/min NA infusions. If the former authors had carried out infusions for longer periods of time a calorigenic effect might have been seen. Also, as mentioned previously, the HP of sheep in the study of Webster *et al.*(1969b) was quite variable even in the absence of catecholamine infusions. An increase in HP of sheep of 30% during NA infusions indicates that the relationship between the NA stimulation of oxygen consumption and the log of body weight (Heldmaier 1971) is not linear over the upper range of body weights.

Adrenaline and NA are assumed to stimulate HP by the same mechamisms in mammals which possess brown adipose tissue (Himms-Hagen 1969) but the calorigenic effect of A is usually less than that of NA (Himms-Hagen 1969; Alexander 1969; Bartunkova *et al.*1971). The sheep of the present study probably had no brown adipose tissue and therefore the most likely origin of A and NA induced HP was skeletal muscle. Adrenaline and NA have been shown to promote skeletal muscle contraction or shivering under a variety of circumstances (Andersson *et al.*1964; Tanche and Therminarias 1969). WA sheep were seen to shiver only during infusions of the highest dose of A and the CA sheep were seen to shiver only in seven of the 24 infusion trials; two of which were saline

infusions. Shivering HP therefore probably was not the main contributor to the calorigenic effect of A and was definately not the cause of NA calorigenesis since shivering was never observed during NA infusions. The contribution to HP of an increase in skeletal muscle tension during A and NA infusions (Bowman and Raper 1967) was not assessed. Newsholm and Crabtree (1976) have proposed that A promotes HP in skeletal muscle by stimulating substrate cycling between glycogen and glucose-1-phosphate or fructose-6-phosphate and fructose 1,6-diphosphate. Nicol *et al.*(1980) have found evidence of increased fructose phosphate cycling in skeletal muscle of cold-acclimated sheep. Also substrate cycling caused by the sympatheticoadrenal medullary system appears to exist in newly hatched domestic fowl (Freeman 1971) where propranolol, a *B*-adrenergic blocking agent, inhibited both cold induced HP and glycogenolysis. In the newborn piglet which has no brown adipose tissue and which responds calorigenically to A but not NA (Leblanc and Mount 1968), propranolol inhibited 60% of cold induced HP (Kaciuba-Uscilko and Ingram 1977). In 6-8 week old pigs propranolol has been shown to block 14% of the normal HP response to moderate cold exposure (Dauncey and Ingram 1979). Recently Christopherson and Kennedy (1980) found that chronic propranolol treatment reduced by 19% the resting HP of shorn sheep acclimated to 10°C and Webster *et al.*(1969b) have shown that four and eight percent, respectively, of the acute cold induced HP of warm- and cold-acclimated sheep is

blocked by propranolol. The magnitude of HP blocked by propranolol in the above studies with sheep is similar to the amount of heat produced by sheep in the present experiment in response to A infusions. Chatonnet (1967) found that adrenal demedullation of spinal dogs reduced peak metabolism in cold environments by 20-30%. Thus A calorigenesis may be important to the dog in severe cold environments. If the calorigenic effect of A in sheep does involve skeletal muscle glucose metabolism, the enhancement of the calorigenic response to A upon cold acclimation of sheep, as shown in the present experiment, may be related to increases in glucose flux and oxidation found during cold-acclimation of sheep (McKay *et al*.1974). Also Graham (1977) has shown that the hyperglycemic response to A is potentiated by cold-acclimation of sheep.

Noradrenaline has been shown to cause partial depolarization of skeletal muscle cell membranes (Teskey *et al*.1975). An increase in the $Na^+/K^+$ ATPase activity in skeletal muscle of cold-acclimated sheep is suggested by the findings of Gregg and Milligan (1980) that ouabain sensitive respiration of sheep skeletal muscle is increased upon cold-acclimation. Since the plasma A and NA levels of sheep exposed to moderate cold are at least double that of warm-exposed sheep (Study 1), the elevated resting HP of cold-exposed sheep may be the result of catecholamine stimulated $Na^+/K^+$ ATPase activity. The increase in the calorigenic effect of A following long term cold-exposure

may be related to either increased $Na^+/K^+$ ATPase activity or substrate cycling at this time.

That cold-acclimation of sheep increased the calorigenic response to A but not NA exemplifies the great difference between the mechanisms involved in the cold-acclimation of sheep and smaller mammals. Humans made mildly thyrotoxic with an oral dose of triiodothyronine, like cold-acclimated sheep, show an increased calorigenic response to A but not NA (Svedmyr 1966). It is suggested that increased thyroid function in cold-acclimated sheep (Westra and Christopherson 1976) may be the means by which tissues of sheep become sensitized to the calorigenic effect of A.

The identical HR response to all doses of A would appear to indicate that the lowest dose was sufficient to stimulate maximally the cardiac *B*-receptors which are thought to mediate this response (Moran 1975). Apparently, cold acclimation does not affect the maximum HR response to A in sheep although it may influence submaximal responses to dose rates lower than those used in the present experiment. In rabbits cold acclimation appears to decrease (Honda *et al*.1962; Carlson 1966) or have no effect (Kockova and Jansky 1968) on the HR response to A. The HR response to isoproterenol, a pure *B*-receptor agonist, is increased upon cold acclimation of the rat (LeBlanc *et al*.1972). The lack of HR response to NA infusion at the two lowest dose rates and the moderate response to the two highest dose rates

probably reflects augmentation of vagal activity to the heart in response to systemic hypertension which is usually caused by NA (Neil 1975).

The lack of a large increase in RT during A and NA infusions agrees with results of Webster *et al.*(1969b) where A and NA doses of 1.0 ug/kg/min had no significant effect on the RT of warm- and cold-acclimated sheep. The quantity of heat produced in response to A and NA in the present experiments would not be expected to result in a large change in RT.

The slightly higher RR of the CA than WA sheep during saline infusions of Experiment I probably reflected the greater need of the former for evaporative cooling because of a higher thermoneutral HP. The greater thermoneutral HP of the CA sheep was also reflected in the greater RT of the CA sheep. The means by which the higher doses of A and all doses of NA caused an increase in RR cannot be ascertained but Joels (1975) has suggested that a similar increase in humans was either a reflex response to increased cardiac output or HP or was a direct effect of A on blood flow to the carotid body.

Webster *et al.*(1969b) found that NA infusions caused a 5°C fall in leg skin temperature when initial skin temperature was above 20°C and room temperature was 10°C. The high (20-23°C) room temperature in the present study may have limited the degree by which vasoconstriction could alter skin temperature during NA infusions.

Adrenaline injections in sheep have been shown to result in rumination and associated reticulo-rumen motility patterns (Kay 1959; Itabisashi 1973). Kay (1959) speculated that the rumination and salivary responses to A are reflex responses to increased neural input from rumen mechanoreceptors sensitized by A. The increases in rumen rhythmic motility sometimes observed during the initial hour of A infusion of Experiment I may have been a manifestation of this reflex. Adrenaline inhibition of rumen motility as found by Habel (1956) was more evident in the WA than CA sheep of the present study. Also inhibition of rumen motility by NA was more evident in the WA than CA sheep of Experiment II. *In vitro* studies (Miert and Huisman 1968; Titchen 1968) have indicated that the inhibition of forestomach smooth muscle contraction by catecholamines is *B*-receptor mediated. Kunos (1977) has provided evidence indicating that the number of myocardial *B*-receptors is reduced in hypothyroid rats. Cold-acclimation of sheep may have a similar effect on rumen smooth muscle *B*-receptors. The greater reticulorumen motility in CA as compared to WA sheep (Westra and Christopherson 1976) may be related to the difference in sensitivity to catecholamine inhibition as shown in the present experiments.

It can be concluded from this study that the catecholamines are calorigenic in sheep and that the calorigenic effect of A is enhanced by chronic cold exposure. Management or environmental stresses which result

in a sympathetic response may, by resulting in an increased HP, cause an additional energy requirement of sheep, particularily if the animal has been exposed to cold for a prolonged period of time. We have also provided evidence that the inhibitory effect of catecholamines on rumen motility is reduced following prolonged cold exposure of sheep.

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## IV. Study 3

Tissue Catecholamine Levels, Noradrenaline Turnover Rate and Organ Weights of Warm- and Cold-acclimated Sheep

### A. Abstract

Concentrations of noradrenaline (NA), adrenaline (A) and dopamine in the tissues of shorn sheep exposed to moderately cold (8-13°C) temperatures for eight weeks were not different from those of sheep exposed to 21 - 24°C. Tissue catecholamine levels in sheep were similar to those of other species and the rumen and proximal colon levels of dopamine were not as high as has been previously reported for other sections of the ruminant gastrointestinal tract. Only the leg skin NA turnover rate, as determined by measuring the decline in tissue NA specific activity following $^{3}$H-NA injection, was greater in cold- than warm-acclimated sheep. A greater organ weight of the reticulorumen and small intestine and digesta weight in the colon of cold- than warm-acclimated sheep was probably the result of the greater feed intake in the former.

## B. Introduction

Elevated levels of noradrenaline (NA) in the plasma of cold-acclimated sheep (Christopherson *et al*.1978, Study 1) suggests that the sympathetic nervous system is activated by cold exposure. However, the detection of a change in plasma NA level gives no indication as to which organs or tissues have altered sympathetic activity and might possibly represent only a change in catecholamine uptake by sympathetic nerve endings or a change in the rate of NA breakdown. It has been suggested that the sympathetic nervous system is involved in numerous physiological responses of sheep to cold such as; elevated heart rate (Webster and Hays 1968), peripheral vasoconstriction (Meyer and Webster 1971) and an increase in reticulorumen contraction rate (Kennedy *et al*.1977). Elevated plasma adrenaline (A) levels of sheep exposed to chronic cold (Study 1) also suggest that an increased adrenal medullary output of catecholamines is sustained throughout long periods of cold exposure.

Sympathetic nervous activity in specific tissues of small mammals under a variety of environmental and pathological conditions has been assessed by estimating the turnover rate of NA (Landsberg and Axelrod 1968; Taubin *et al*.1972; Djahanguire *et al*.1973; Tu and Nash *et al*.1974; Young and Landsberg 1977; Tanaka *et al*.1977). The technique involves injection of $^{3}$H-noradrenaline ($^{3}$H-NA) which equilibrates with endogenous NA stores in sympathetic nerve

endings. The subsequent release of labelled NA from tissues is proportional to the degree of sympathetic activity (Hertting and Axelrod 1961) and the rate of decrease in NA specific activity in a tissue is an index of NA turnover (Costa *et al*.1966).

The present study is the first for which the above method of assessing sympathetic activity has been applied to large animals. We examined the endogenous concentrations of NA, A and dopamine (DA) and the NA turnover in various tissues of warm-acclimated (WA) and cold-acclimated (CA) sheep. The weights of vital organs and some digestive parameters of WA and CA sheep were also compared.

## C. Material and Methods

Twelve wethers fourteen months of age were used. Ten wethers which had been used for NA infusions in Study 2 were maintained in the warm (21-24°C) or cold (4-5°C) environments for an additional maximum eight day period for the purpose of the present trial. One additional animal had also been maintained in each environment for the eight week period but these sheep had not received NA infusions. As in the 8 week cold-acclimation period, sheep at 21-24°C received 700 g and sheep at 4-5°C received 1100 g good quality alfalfa pellets twice daily. Water and cobalt iodized salt were freely available and room lighting was continuous. Fleece length was approximately 2 cm and mean±SEM body weights for the WA and CA sheep were 46.5±2.9 and 46.0±1.7 kg, respectively. Jugular catheters inserted during the eighth week of cold-acclimation were retained for the present trial.

D,L-noradrenaline-7-$^{3}$H (15 Ci/mmol) obtained from New England Nuclear Corp. was diluted 2:5.5 (v:v) with acidified saline just prior to injection and was administered by way of a jugular catheter at a dosage of 16.3 $\mu$Ci/kg. On days 2-7 one WA and one CA sheep were injected daily between 845 h and 1215 h. Three each of the warm and CA sheep were sampled 1.75 h following tracer injection and the remainder were sampled 24 h post-injection. The sheep were fed 2 h prior to tracer injection. One h prior to injection jugular catheters were extended through portholes to the outside of

the animal room so that the sheep were undisturbed for one h prior to and at least one h post-injection of tracer. For sheep sampled 24 h post-injection of tracer, feed and water were offered at 1700 h on the day of injection and three h prior to sampling the following day. Jugular catheters were secured to the neck area overnight and were again extended out of the animal room two h prior to sampling. To prevent excessive sympathetic activity prior to tissue sampling, intravenous nembutal (Abbott) at a dose of 16 mg/Kg was administered through the room exteriorized jugular catheter. The anesthetized animal was then moved to a surgical area where anesthesia was maintained during tissue sampling.

Duplicate 3-4 g tissue samples were taken over a period of 45 min and the animal was then killed by removal of the heart. The tissue sampling sequence was as follows; leg skin (metacarpal region), ear skin (medial dorsal region), and through a midline incision; colon (proximal region), rumen (anterior of ventral sac), adrenal glands, heart ventricle, heart atrium. Tissue subsamples (0.5-1.0 g) were frozen and later assayed for dry matter (DM) and nitrogen content (Kjeldahl method, Association of Official Analytical Chemists 1975). After excision, skin and heart samples were immediately placed in plastic vials and frozen in liquid nitrogen. On a cold stainless steel surface the muscle layers of the colon and rumen samples were separated from the underlying tissue (mucosa) and the adrenal medullae (0.27-0.95g) were carefully separated from the cortex prior

to freezing in liquid nitrogen. One of the WA sheep appeared to have only one adrenal gland.

Weights of the liver, heart and kidneys were recorded and the various sections of the gastrointestinal tract were weighed with and without contents. The digesta DM was determined by heating at 95°C to a constant weight.

On the same day as sampling, tissues for $^{3}$H-NA and catecholamine analysis were partially thawed, weighed and homogenized with a Polytron homogenizer (Brinkman Inst. Co.). Samples were homogenized on ice in 10 ml of ice-cold 0.1N perchloric acid containing 5 mM EGTA and 5 mM glutathione. Following centrifugation for 20 min at 1°C and 20,000 X G the bulk of the supernatant was stored in plastic vials at -40°C until $^{3}$H-NA could be determined. A 0.5 ml aliquot of supernatant of one of the duplicate tissue samples was diluted to 10 ml with cold perchloric acid solution and was stored at -40°C until endogenous catecholamine assay was carried out. NA was extracted from tissue supernatant as described by Diamant and Byers (1975) except that the batch alumina method was employed and 3,000 d.p.m. of alumina purified D,L-noradrenaline-7-$^{14}$C (52.3 mCi/mmol) (NEN) was added to supernatants prior to extraction such that $^{3}$H-NA values could be corrected for recovery. Dual label liquid scintillation counting of the 0.5N acetic acid eluates in 10 ml of Aqueous Counting Scintillant (Amersham) was done with a Mark IV Liquid Scintillation Counter (Nuclear Chicago, Searle Analytic

Inc., Des Plaines, Ill.). No attempt was made to separate adrenal A and NA prior to determination of tissue activity. Because NA is an A precursor, some of the radioactivity of the adrenal must have been present in the form of $^{3}$H-A as well as $^{3}$H-NA. The proportion of total radioactivity present in association with adrenaline would have been negligible for sheep killed at 1.75 h but may have been substantial for those sheep killed at 24 h.

NA, A and DA content of the dilute tissue supernatant was measured by the radioenzymatic technique of Peuler and Johnson (1977) with minor modifications as previously described (Study 1). Also, prior to analysis, the 50 $\mu$l perchloric acid samples were neutralized with 15 $\mu$l of 0.25N NaOH.

Specific activity (SA) of tissue NA was logarithmically transformed and the regression coefficient (-k) for the relationship between lnSA and time after injection of tracer was calculated by linear regression analysis (Steel and Torrie 1960). Turnover time (1/k), half-life (0.693/k) and turnover rate (k X endogenous NA concentration) ± 95% confidence intervals (C.I.) were calculated as indicated by Djahanguiri *et al.*(1973). Because a substantial proportion of adrenal radioactivity was probably labelled adrenaline in the sheep killed at 24 but not 1.75 h, the estimates for adrenal medullary NA fractional turnover rate and turnover rate are underestimates and for half-life and turnover time are overestimates.

Data were analyzed using analysis of variance and where significant differences existed means were compared using the Student-Newman-Keuls' test of mean differences (Steel and Torrie 1960). Slopes (fractional turnover rates) were compared using the Student t test (Steel and Torrie 1960).

**D. Results**

There was no effect of temperature of acclimation on endogenous concentrations of NA, A and DA or on the fractional turnover rate and half-life of NA in the various tissues. Therefore, values presented below have been calculated by pooling the data of the two temperature treatment groups.

When expressed in terms of ug/g tissue wet weight or ug/g tissue DM (Table 8) the endogenous NA and A mean concentrations in the heart ventricle were greater than respective levels in all other non-adrenal tissues. The mean±SEM DA concentration (ug/g tissue) of the ear skin (0.37±0.10) was greater ($P<0.05$) than that of the leg skin (0.15±0.05), rumen muscle (0.08±0.02) and rumen mucosa (0.07±0.02). If expressed as ug DA/g DM, only the DA concentration of the proximal colon mucosa (2.81±0.87) was greater ($P<0.05$) than in other non-adrenal tissues. The mean±SEM adrenal medulla concentration of DA (146±50 ug/g) was much lower than either that of A (1807±614 ug/g) or NA (1016±253 ug/g). Tissue catecholamine levels expressed in terms of ug catecholamine/g nitrogen have not been included in Table 8 as differences between tissues on a per g nitrogen basis were identical to differences found on a per g DM basis.

The NA fractional turnover rates (k) (Table 9) in the ear skin (0.084 $h^{-1}$), proximal colon muscle (0.057 $h^{-1}$) and rumen mucosa (0.065 $h^{-1}$) were similar and did not differ

Table 8. Tissue concentrations of noradrenaline (NA), adrenaline (A) and dopamine (DA) expressed /g wet weight and /g dry matter (DM) (Mean ± SEM*)

| | Concentration | | | | | |
|---|---|---|---|---|---|---|
| | NA | | A | | DA | |
| Tissue | µg/g tissue wet wt. | µg/g tissue DM | µg/g tissue wet wt. | µg/g tissue DM | µg/g tissue wet wt. | µg/g tissue DM |
| Leg skin | 0.15[a†] ±0.05 | 0.61[a] ±0.23 | 0.08[a] ±0.04 | 0.35[a] ±0.18 | 0.15[a] ±0.05 | 0.61[a] ±0.21 |
| Ear skin | 0.13[a] ±0.03 | 0.50[a] ±0.12 | 0.23[a] ±0.15 | 0.85[a] ±0.55 | 0.37[b] ±0.10 | 1.38[a] ±0.33 |
| Proximal colon (muscle) | 0.09[a] ±0.02 | 0.53[a] ±0.14 | 0.08[a] ±0.03 | 0.48[a] ±0.18 | 0.22[ab] ±0.05 | 1.24[a] ±0.38 |
| Proximal colon (mucosa)÷ | 0.12[a] ±0.04 | 1.03[a] ±0.32 | 0.17[a] ±0.07 | 1.78[a] ±0.70 | 0.29[ab] ±0.07 | 2.81[b] ±0.87 |
| Rumen (muscle) | 0.08[a] ±0.01 | 0.45[a] ±0.05 | 0.06[a] ±0.02 | 0.36[a] ±0.13 | 0.08[a] ±0.02 | 0.43[a] ±0.10 |
| Rumen (mucosa) | 0.13[a] ±0.02 | 0.72[a] ±0.14 | 0.14[a] ±0.06 | 0.82[a] ±0.32 | 0.07[a] ±0.02 | 0.42[a] ±0.11 |
| Heart ventricle | 2.41[b] ±0.78 | 13.09[b] ±4.17 | 2.02[b] ±0.58 | 11.64[b] ±3.59 | 0.24[ab] ±0.07 | 1.36[a] ±0.26 |
| Heart atrium | 1.10[a] ±0.23 | 5.26[a] ±1.00 | 0.36[a] ±0.07 | 1.73[a] ±0.28 | 0.18[ab] ±0.02 | 0.86[a] ±0.10 |
| Adrenal medulla | 1016 ±253 | | 1807 ±614 | | 146 ±50 | |

* Standard error of mean.

÷ Includes submucosa.

† Means within a column with a common superscript were not significantly different (adrenal values not included in the comparison of means) (P>0.05).

Table 9. Fractional turnover rate, turnover time, half-life and turnover rate of noradrenaline in tissues of sheep†

| Tissue | Fractional turnover rate (k) | Turnover time (h) | Half-life (h) | Turnover rate (µg/g/h) |
|---|---|---|---|---|
| Leg skin | 0.119[be] ±0.028* | 8.4 ±2.0 | 5.8 ±1.4 | 0.018[ad] (0.011-0.025)‡ |
| Ear skin | 0.084[acde] ±0.034 | 11.9 ±4.8 | 8.3 ±3.4 | 0.011[a] (0.006-0.017) |
| Proximal colon (muscle) | 0.057[acde] ±0.033 | 17.5 ±10.1 | 12.2 ±7.1 | 0.005[a] (0.002-0.008) |
| Proximal colon (mucosa)÷ | 0.104[bc] ±0.033 | 9.6 ±3.0 | 6.7 ±2.1 | 0.012[a] (0.006-0.018) |
| Rumen (muscle) | 0.108[bd] ±0.016 | 9.3 ±1.4 | 6.4 ±1.0 | 0.009[a] (0.008-0.010) |
| Rumen (mucosa) | 0.065[acde] ±0.026 | 15.4 ±6.2 | 10.7 ±4.3 | 0.008[a] (0.005-0.010) |
| Heart ventricle | 0.039[ac] ±0.019 | 25.6 ±12.5 | 17.8 ±8.7 | 0.094[bd] (0.021-0.167) |
| Heart atrium | 0.051[ace] ±0.022 | 19.6 ±8.5 | 13.6 ±5.9 | 0.056[b] (0.030-0.082) |
| Adrenal medulla | 0.022[a] ±0.015 | 45.6 ±31.1 | 31.5 ±21.5 | 22.350[c] (4.250-40.450) |

† Values for warm and cold-acclimated sheep have been combined.

÷ Includes submucosa.

* Standard error of mean.

a,b,c,d,e Means within a column with a common superscript did not differ significantly (P>0.05).

‡ 95% confidence interval.

from rates in any other tissues. The leg skin (0.119 $h^{-1}$) and rumen muscle (0.108 $h^{-1}$) fractional turnover rates were greater ($P<0.05$) than the rates found in the heart ventricle (0.039 $h^{-1}$) and adrenal medulla (0.022 $h^{-1}$). Also the fractional turnover rate in the heart atrium (0.051 $h^{-1}$) was less ($P<0.05$) than in rumen muscle and that in the colon mucosa was greater ($P<0.05$) than in the adrenal medulla. Differences between NA turnover rates (*u*g/g/h) were considered significant ($P<0.05$) if the 95% C.I. did not overlap (Taubin *et al*.1972). NA turnover rate (Table 9) in the adrenal medulla (22.35 *u*g/g/h) was greater than in the heart ventricle (0.094 *u*g/g/h) and the heart atrium (0.056 *u*g/g/h) and turnover rates in the adrenal medulla and heart atrium were greater than in all skin and gut tissues. Turnover rate in the heart ventricle was greater than in ear skin and all gut tissues.

Although temperature of acclimation generally had no significant effect on tissue NA levels and kinetics, the turnover rate ± 95% C.I. of NA in the leg skin of CA sheep (0.028±0.002 *u*g/g/h) was greater ($P<0.05$) than that of the leg skin of WA sheep (0.009±0.008 *u*g/g/h). Both nonsignificantly greater endogenous NA concentration and fractional turnover rate in the CA than WA sheep leg skin contributed to the difference in turnover rate.

Table 10 contains mean±SEM organ weights and digesta total weight, DM and water weights for various sections of the gastrointestinal tract and the mean±SEM heart, liver,

Table 10. Organ and organ content (water and dry matter (DM)) weights for warm-acclimated (W) and cold-acclimated (C) sheep

| | | Weight (g) | | | | |
|---|---|---|---|---|---|---|
| Organ | Treatment | Organ + contents | Organ | Contents | Contents water | Contents DM |
| Reticulo-rumen | W | 9,342±380 * | 856±21$^{i}$ | 8,486±363 | 7,026±323 | 1,460±46 |
| | C | 9,691±625 | 1,013±22$^{j}$ | 8,677±614 | 7,067±518 | 1,610±118 |
| Abomasum | W | 723±68 | 264±21 | 469±62 | 406±55 | 53±9 |
| | C | 554±64 | 245±11 | 308±64 | 278±57 | 31±7 |
| Small intestine | W | 1,385±43 | 581±21$^{a}$ | 804±30 | 704±27 | 100±5 |
| | C | 1,420±50 | 725±40$^{b}$ | 694±59 | 609±50 | 86±9 |
| Colon | W | 843±58$^{g}$ | 363±18 | 480±62$^{c}$ | 343±53$^{c}$ | 136±15$^{e}$ |
| | C | 1,138±42$^{h}$ | 397±10 | 742±41$^{d}$ | 545±31$^{d}$ | 197±14$^{f}$ |
| Cecum | W | 1,161±109 | 186±19 | 975±102 | 808±85 | 167±18 |
| | C | 1,359±102 | 211±25 | 1,147±80 | 962±67 | 185±16 |
| Total gut | W | 13,453±258 | 2,250±44$^{i}$ | 11,204±251 | 9,287±204 | 1,916±107 |
| | C | 14,162±640 | 2,591±58$^{j}$ | 11,568±549 | 9,461±526 | 2,109±105 |
| Omasum | W | | 463±33 | | | |
| | C | | 528±79 | | | |
| Liver | W | | 612±17$^{g}$ | | | |
| | C | | 757±33$^{h}$ | | | |
| Kidney | W | | 143±10 | | | |
| | C | | 158±8 | | | |
| Heart | W | | 200±7 | | | |
| | C | | 191±7 | | | |

* Mean ± standard error.

abc Means within a column within an organ with different superscripts were different at the following probability levels; ab <0.05, cd <0.01, ef <0.025, gh <0.005, ij <0.001.

kidney and omasal weights of the WA and CA sheep. Omasal digesta weight was not recorded. All organs except the abomasum and heart of the CA sheep were heavier than for the WA sheep but the effect of temperature treatment was significant only for the reticulorumen ($P<0.001$), small intestine ($P<0.05$) and liver ($P<0.005$). The weight of the entire gastrointestinal tract plus contents was similar in the two treatment groups but the total mean±SEM weight of the empty gastrointestinal tract of the CA sheep (2.59±0.06 kg) was greater ($P<0.001$) than that of the WA sheep (2.25±0.04 kg). The colon of the CA sheep contained 59% greater ($P<0.01$) water and 44% greater ($P<0.025$) DM than the colon of the WA sheep. In all other sections of the gastrointestinal tract the DM and water content was not influenced by temperature treatment.

## E. Discussion

The level of NA in the heart ventricle was slightly greater than that found previously (Goodall 1951; Bertler *et al*.1959; Bertler 1961) for sheep hearts (1.12 - 2.20 *u*g/g). Previous assays may have included atrial tissue which contained less NA than ventricular tissue in the present study. The level of NA in the hearts of other mammals (Shore *et al*.1958; Jarrot 1970; Tu and Nash 1974; Tanaka *et al*.1977) is comparable to that of sheep. Tissue level of NA reflects degree of sympathetic innervation (Euler and Lishajko 1957; Potter *et al*.1965; Kopin 1965) since a negligible amount of NA is located extra-neuronally and NA synthesis increases to replace lost NA when sympathetic activity is increased (Roth *et al*.1966; Gordon *et al*.1966). Thus, degree of sympathetic innervation of the heart appears similar in most species. That cold acclimation did not affect tissue NA concentration probably indicates that the degree of sympathetic innervation of the various tissues was not altered by prolonged cold exposure.

The level of A found in the sheep heart ventricle was much higher than that previously found for sheep (Goodall 1951) and rabbit (Angelakos *et al*.1963) heart (0.12 *u*g/g) and for the rat heart (0.01 *u*g/g) (Pendleton *et al*.1978) but was similar to that in the golden hamster heart (0.67 *u*g/g) (Lew and Quay 1973). The activity of phenylethanolamine N-methyltransferase (PNMT), the enzyme which converts NA to A, is usually low in extra-adrenal tissue such as the rat

heart (Pendleton *et al*.1978). Low activity of PNMT in extra-adrenal tissue and, therefore, low levels of A are believed to be due to the abscence of high levels of glucocorticoids at such locations (Wurtman 1966). The high level of A found in the sheep and golden hamster heart suggests that heart PNMT activity may be greater in the hearts of these species than in the rat heart. As found presently, in rabbits the level of A in the heart ventricle is greater than in the atrium (Angelakos *et al*.1963).

The level of DA found in the sheep proximal colon, although greater than that seen in some other tissues, was much lower than levels (1.2 - 7.6 *u*g/g) previously found (Bertler *et al*.1959; Schumann and Heller 1959; Bertler 1961, Nasmyth 1967) in the small intestine and colon of sheep. It appears that the proximal colon and rumen (present study) like the abomasum (Bertler *et al*.1959) of sheep have a relatively low number of DA containing mast cells. Mast cells are the structures which contain the majority of the DA found in other sections of the ruminant gastrointestinal tract (Falck *et al*.1964). It is improbable that the assay technique for DA failed to detect actual high levels of DA present in the gut since a high level of DA in the adrenal medulla (146 *u*g/g) was demonstrated.

As found in the present study, heart DA level is usually less than 15% of that of NA (Angelakos *et al*.1963; Costa *et al*.1973; Snider *et al*.1973).

Adrenal medullary levels of A and NA were similar to

levels found in the rat adrenal (Lew and Quay 1973; Tu and Nash 1974; Roy *et al*.1977) and the sheep adrenal (Goodall 1951; Comline and Silver 1961) considering that in previous studies levels were expressed per g of whole adrenal rather than per g of medullary tissue. The low adrenal medullary content of DA was expected in that DA is a precursor in NA synthesis and dopamine *B*-hydroxylase is not a rate limiting enzyme in the synthesis of NA (Snider *et al*.1973).

Criticism of the present calculation of fractional turnover rates may be made in that SA was measured at only two time periods and therefore the relationship between lnSA and time was assumed to be linear (Appendix Table 1). Such a linear relationship has been proven in the rat where Costa *et al*.(1966) showed that following $^{3}$H-NA injection the label content of tissues declined in a single exponential fashion over a period of two minutes to 40 h. This linear relationship has been repeatedly demonstrated (Landsberg and Axelrod 1968; Taubin *et al*.1972). A diphasic decline in lnSA, indicating the presence of two distinct NA pools, was found (Beaven *et al*.1963) only when the amount of NA injected was in excess of a tracer amount (Costa *et al*.1966). It has been demonstrated by Costa *et al*.(1966) that a NA free base dose of 0.165 *u*g/kg is a tracer amount for which a single exponential decline in SA is found. In the present study the NA free base dose injected with $^{3}$H-NA was 0.18 *u*g/kg.

The tendency for NA fractional turnover rate to be the

lowest in the heart and adrenal tissues agrees with previous literature. Taubin *et al.*(1972) found that the NA fractional rate in the rat heart (0.068 $h^{-1}$) was less than in the small intestine (0.093 $h^{-1}$) and colon (0.102 $h^{-1}$) and that of the rat adrenal medulla (Udenfriend and Wyngaarden 1956) is probably quite low since adrenal A fractional turnover rate has been found to be 0.0041 $h^{-1}$. The present data for sheep imply that under the conditions of both warm and cold exposure the skin and gut sympathetic nerves released a greater proportion of stored NA than did heart sympathetic nerves and adrenal chromaffin cells. As found in the present study with sheep, Taubin *et al.*(1972) found the NA turnover rate in the rat heart (0.051 *u*g/g/h) was greater than in gut tissues (0.011 - 0.035 ug/g/h). It therefore appears that rate of NA synthesis in the heart of sheep, like that of the rat, is greater than in gastrointestinal tissue. Also the rate of NA synthesis in the skin of sheep appears to be less than in heart tissue. The greater turnover rate of NA in the leg skin of CA compared to WA sheep reflects the involvement of the sympathetic nervous system in the vasoconstriction response to cold (Meyer and Webster 1971). Supporting this, Hills (1979) has shown that the NA efflux from the ear of an anesthetized sheep increased when the ear vasoconstricted while submerged in an ice bath. The turnover rate of NA in the adrenal medulla (22.35 *u*g/g/h) was remarkably similar to the sheep adrenal medullary output of NA (15.4 *u*g/g/h) calculated from the data of Comline and Silver (1961) with

the assumption that the adrenal medulla weight in the latter study was the same as the mean weight (0.72 g) in the present study. Comline and Silver (1961) measured directly adrenal NA output 15-30 minutes following splanchnic nerve section. As stated in Material and Methods, the adrenal medulla NA turnover rate calculated in the present study is an underestimate since the adrenal radioactivity associated with A was treated as $^{3}$H-NA.

That cold acclimation had no significant effect on NA kinetic parameters in tissues other than the leg skin indicates that the effects of temperature, if any, were too small to be detected using the present assay techniques and number of animals. Differences might have been seen if the cold exposure had been acute since Rubenson (1969) has shown that various short term stresses such as immobilization and electric shock increased rat heart sympathetic activity (as measured by the disappearance rate of $^{3}$H-NA).

The increase in gastrointestinal weight (particularily the reticulorumen and small intestine) associated with long term cold exposure was probably related to the greater feed intake in the cold, rather than to cold *per se*. Tulloh (1966) found that the weight of the gastrointestinal tract is greater in grazing lactating than non-lactating cows. The increase in weight presumably was a response to a greater *ad libitum* intake during lactation. Tulloh (1966) also found that the lactating cows had greater quantities of digesta in all segments of the gastrointestinal tract. In the present

study only the colon of the CA sheep contained more digesta than that of the WA sheep. It is probable that cold-induced reduction in reticulorumen transit time (Kennedy and Milligan 1978) prevented accumulation of digesta in the reticulorumen.

Assessment of sympathetic nervous activity using $^{3}$H-NA disappearance curves appears possible in sheep but the large variability between animals indicates that large numbers may be needed to detect relatively small shanges in sympathetic nervous activity. The turnover rate of NA in the leg skin of CA sheep is greater than in the leg skin of WA sheep. This reflects the involvement of the sympathetic nervous system in the vasoconstriction of skin and suggests that some of the increase in circulating NA in CA sheep (Study 1) may arise from sympathetic nerves in the skin. Tissue levels of NA, A and DA were usually similar to that previously reported for sheep and other species but a very high level of DA which has been previously shown in some portions of the ruminant gastrointestinal tract was not found in the rumen and proximal colon of the sheep of the present study.

The increased maintenance feed requirement of CA sheep appears to result in hypertrophy of the reticulorumen and small intestine although it was not possible to rule out the possibility in the present study that the hypertrophy was a direct effect of cold.

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Table 1. Tissue noradrenaline specific activity (S.A.) and lnS.A. 1.75 and 24 hours post-injection of $^{3}$H-noradrenaline and regression parameters for sheep exposed to chronic warm (W) and cold (C) temperatures

| | | Time (hours) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1.75 | | 24.00 | | Regression Analysis | | | |
| Tissue | Treatment | S.A. (nCi/µg) † | lnS.A. | S.A. (nCi/µg) | lnS.A. | b | $s_b$ | r | (P<) |
| Leg skin | W | 35.9±15.4* | 3.58 | 2.9± 1.6 | 1.06 | 0.109 | 0.038 | 0.82 | 0.05 |
| | C | 10.4± 4.3 | 2.34 | 0.6± 0.2 | -0.51 | 0.128 | 0.028 | 0.92 | 0.01 |
| | $\bar{X}$ | 23.2± 9.2 | 3.14 | 1.8± 0.9 | 0.59 | 0.119 | 0.028 | 0.80 | 0.005 |
| Ear skin | W | 26.4±22.7 | 3.27 | 2.7± 1.8 | 0.99 | 0.096 | 0.061 | 0.67 | NS |
| | C | 5.7± 1.7 | 1.74 | 1.5± 0.8 | 0.41 | 0.080 | 0.044 | 0.67 | NS |
| | $\bar{X}$ | 13.9± 8.8 | 2.63 | 2.1± 0.9 | 0.74 | 0.084 | 0.034 | 0.63 | 0.05 |
| Proximal colon (muscle) | W | 91.7±53.3 | 4.52 | 68.0±54.8 | 4.22 | 0.035 | 0.051 | 0.32 | NS |
| | C | 75.7± 8.9 | 4.33 | 36.2±30.4 | 3.59 | 0.079 | 0.049 | 0.63 | NS |
| | $\bar{X}$ | 83.7±24.4 | 4.43 | 52.1±28.9 | 3.95 | 0.057 | 0.033 | 0.48 | NS |
| Proximal colon (mucosa) ‡ | W | 135.1±27.3 | 4.91 | 64.5±60.8 | 4.17 | 0.110 | 0.067 | 0.63 | NS |
| | C | 76.8±11.1 | 4.34 | 12.5± 6.9 | 2.53 | 0.097 | 0.029 | 0.85 | 0.025 |
| | $\bar{X}$ | 106.0±18.5 | 4.66 | 38.5±29.7 | 3.65 | 0.104 | 0.033 | 0.70 | 0.025 |
| Rumen (muscle) | W | 96.3±13.3 | 4.57 | 13.8± 6.7 | 2.62 | 0.097 | 0.023 | 0.90 | 0.01 |
| | C | 113.6±51.2 | 4.73 | 6.5± 0.9 | 1.87 | 0.118 | 0.026 | 0.92 | 0.01 |
| | $\bar{X}$ | 105.0±24.0 | 4.65 | 10.1± 3.4 | 2.31 | 0.108 | 0.016 | 0.90 | 0.001 |
| Rumen (mucosa) | W | 174.6±46.7 | 5.16 | 57.6±40.2 | 4.05 | 0.069 | 0.035 | 0.70 | NS |
| | C | 59.7±31.0 | 4.09 | 13.6± 5.3 | 2.61 | 0.061 | 0.028 | 0.74 | 0.10 |
| | $\bar{X}$ | 117.1±35.9 | 4.76 | 35.6±20.6 | 3.57 | 0.065 | 0.026 | 0.62 | 0.05 |
| Heart ventricle | W | 29.4± 9.9 | 3.38 | 19.7± 4.7 | 2.98 | 0.015 | 0.019 | 0.35 | NS |
| | C | 26.0± 4.7 | 3.26 | 8.0± 3.7 | 2.08 | 0.064 | 0.027 | 0.76 | 0.10 |
| | $\bar{X}$ | 27.7± 4.9 | 3.32 | 13.8± 3.7 | 2.62 | 0.039 | 0.019 | 0.55 | 0.10 |
| Heart atrium | W | 24.0±10.3 | 3.18 | 12.8± 0.8 | 2.55 | 0.018 | 0.023 | 0.37 | NS |
| | C | 58.3±34.1 | 4.07 | 5.7± 2.4 | 1.74 | 0.093 | 0.038 | 0.81 | 0.10 |
| | $\bar{X}$ | 41.1±17.7 | 3.72 | 9.9± 1.9 | 2.29 | 0.051 | 0.022 | 0.61 | 0.05 |
| Adrenal medulla | W | 88.1±29.0 | 4.48 | 70.1±42.1 | 4.25 | 0.018 | 0.025 | 0.34 | NS |
| | C | 99.2±42.0 | 4.60 | 52.4±22.7 | 3.96 | 0.028 | 0.021 | 0.56 | NS |
| | $\bar{X}$ | 93.6±16.4 | 4.54 | 61.5±15.6 | 4.12 | 0.022 | 0.015 | 0.43 | NS |

† pCi/µg in adrenal medulla.
* Standard error of mean.
NS Not significant (P>0.10) b value.
‡ Includes submucosa.
$\bar{X}$ Mean value for W and C sheep.

## V. Conclusions

An effect of chronic cold exposure on thermoneutral heat production was consistantly demonstrated. For sheep exposed for at least four weeks to moderate cold in Study 1, and during the adrenaline and noradrenaline infusion trials of Study 2, the increases in thermoneutral heat production per °C lowering of room temperature ($kJ/kg^{0.73}/min/°C$) were 0.00394, 0.00494 and 0.00357, respectively. Temperatures in the cold environments were 15.5, 8.5 and 14.0 °C, respectively, below the temperatures in the warm environments. The comparable value for the intermittent cold exposed sheep of Study 1 was 0.00387 $kJ/kg^{0.73}/min/°C$. The above values represent a mean 1.84% increase in thermoneutral heat production per °C lowering of acclimation temperature. The results indicate that in sheep there is at least one common characteristic of acclimation to constant and intermittent cold exposure.

Adrenaline and noradrenaline were calorigenic in both warm- and cold-acclimated sheep. The response to adrenaline but not noradrenaline was enhanced by previous cold acclimation. Because plasma catecholamine levels were found to be elevated throughout a prolonged period of constant cold exposure and during the warm daytime periods for the intermittent cold exposed sheep, it appears that catecholamine calorigenesis may be an important factor in terms of both the heat production response of sheep to cold and the elevation in thermoneutral heat production seen in

cold-acclimated sheep. In addition it is possible that high circulating levels of catecholamines in some manner result in tissue sensitization with respect to the calorigenic effect of adrenaline. The increased sensitivity of cold-acclimated sheep to the calorigenic effect of adrenaline suggests that cold-acclimated sheep may be subject to an extra energy expenditure under any management or environmental stress which evokes adrenalmedullary secretion of adrenaline.

Catecholamine calorigenesis in sheep did not appear to be associated with skeletal muscle contraction and therefore it was suggested that the calorigenic response of sheep to catecholamines was a non-shivering response in skeletal muscle. Cortisol metabolism was elevated in sheep throughout a 50 day period of moderate cold exposure. The possibility exists that the increased cortisol metabolism contributed to the sensitization of sheep to the calorigenic effect of adrenaline.

Changes in heart rate during catecholamine infusions did not correlate well with changes in heat production. Thus it is concluded that heart rate is only a poor index of heat production.

Catecholamine inhibition of rumen motility appeared to be supressed by cold acclimation. This adjustment could promote increased reticulorumen motility in cold-acclimated sheep.

Noradrenaline turnover studies in sheep require more

animals than used in the present study where only a difference in skin sympathetic activity could be demonstrated between warm- and cold-acclimated animals. No differences in tissue catecholamine levels were found when sheep acclimated to 9 weeks of cold and warm temperatures were compared. Excessive tissue dopamine levels were not found in the rumen and colon as has been found in other sections of the ruminant gastrointestinal tract.

Greater feed intake in the cold environment was probably the cause of increased reticulorumen and small intestine weights and increased fill in the colon of cold-acclimated sheep but from the results of the present study a more direct effect of cold on gastrointestinal weight and weight of gut fill cannot be disproved.